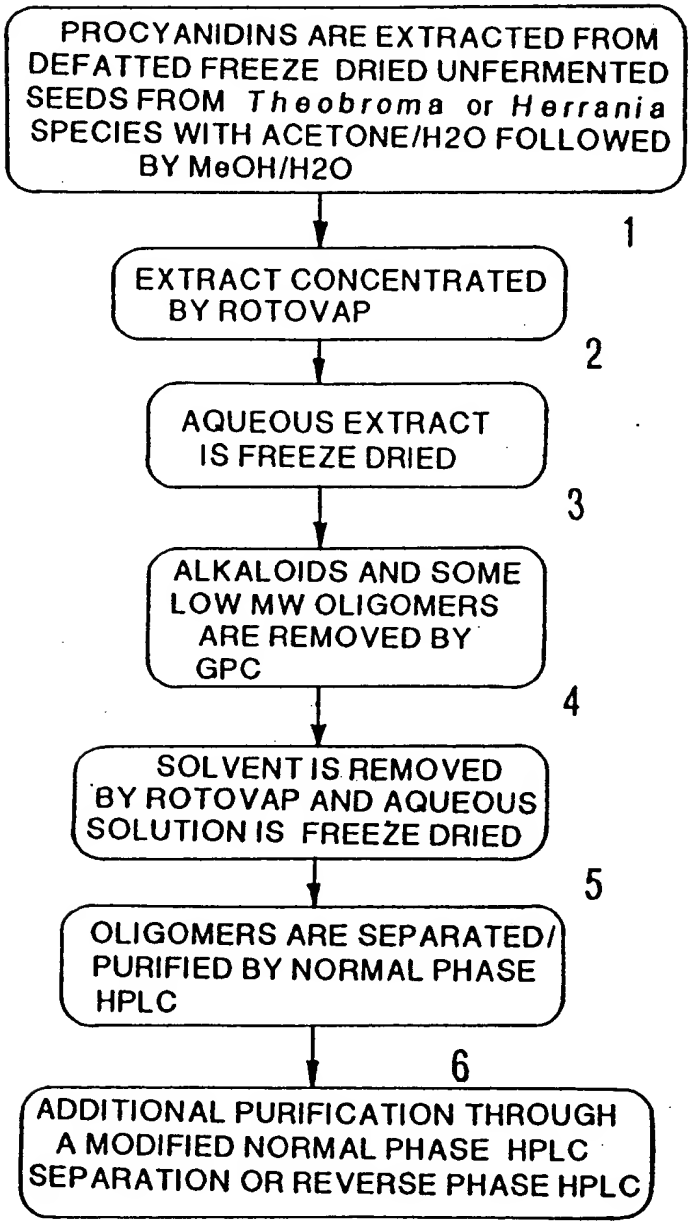
FIG.1

Summary of the current purification protocol



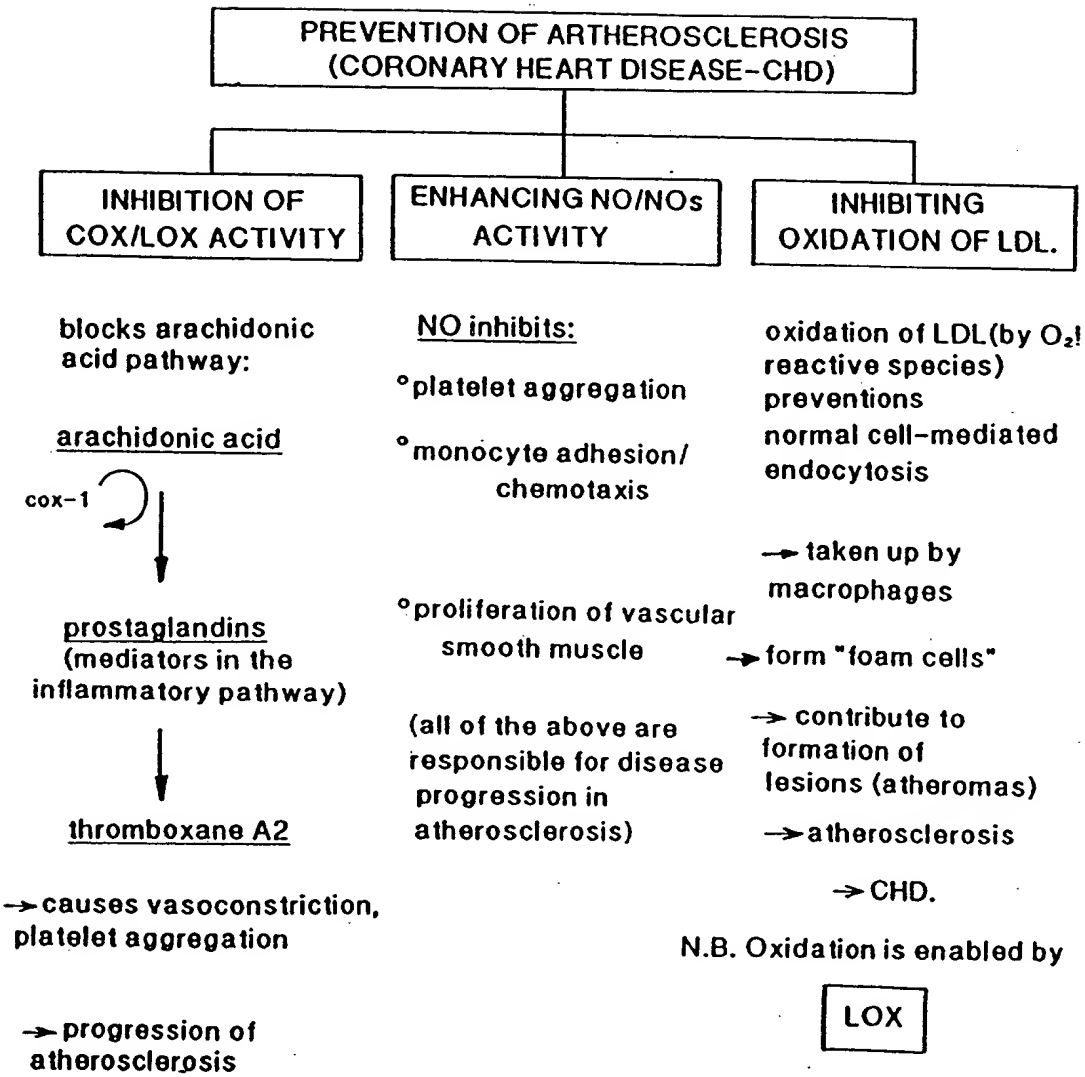
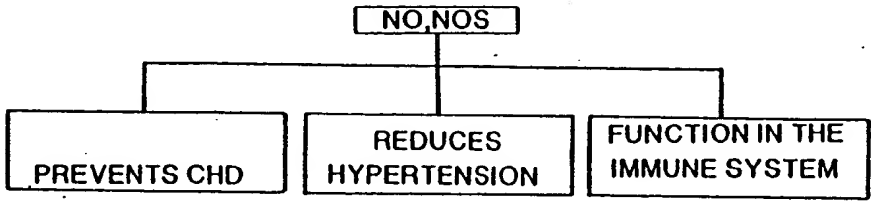


Chart showing the major contributing factors in the progression of Colonary Heart Disease (CHD) and how the activity of cocoa procyanidins contributes to the prevention of the progression of the disease state

FIG.2 a

The cocoa procvanidins induce the activity of NOS and therefore the resulting production NO, thereby enhancing the health benefits mediated by the activity of nitric oxide (NO).



°inhibits platelet aggregation, monocyte adhesion, chemotaxis and vascular smooth muscle proliferation thereby causing vascular relaxation and preventing the disease progression of CHD.

By lowering blood pressure via the following mechanism:

vascular endothelial cells release eNOS

→result in production of NO

→NO relaxes vascular smooth muscles, increasing vascular lumen diameter

→lowers blood pressure

→induces hypotension.

HYPERTENSION RESPONSIBLE FOR CARDIOVASCULAR DISEASES:

including:

stroke
heart attack
heart failure
kidney failure

° Macropages have a different NOS(iNOS)

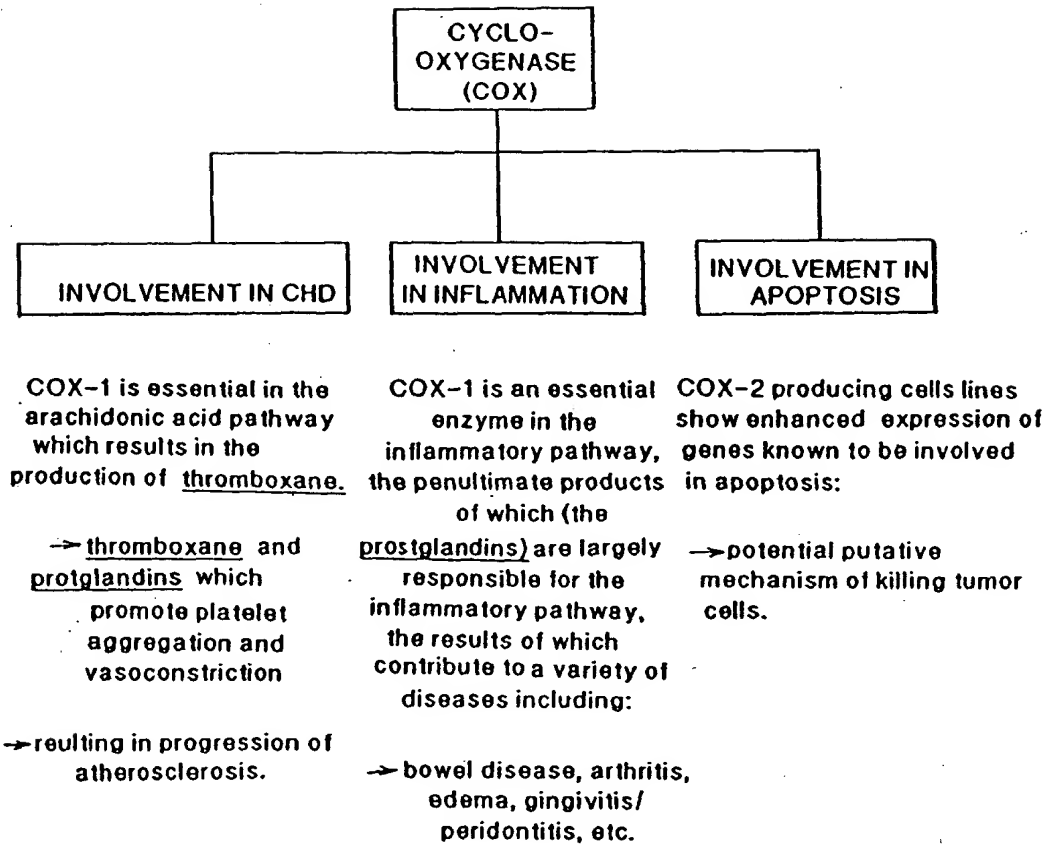
° iNOS gene transcription is controlled by cytokines

° iNOS activity results in macrophage NO production at sufficient concentrations to inhibit ribonnclease reductase

→causes inhibition of DNA synthesis

→potential mechanism of action in anti-tumor and anti-microbial function.

FIG.2b



The cocoa procyanidins inhibit the production of cyclo-oxygenase, thereby blocking the arachidonic acid pathway, which is responsible for the inflammatory response and the vasoconstrictive and platelet aggregating responses which contribute to the disease progression of CHD.

FIG.2c

FIG.3

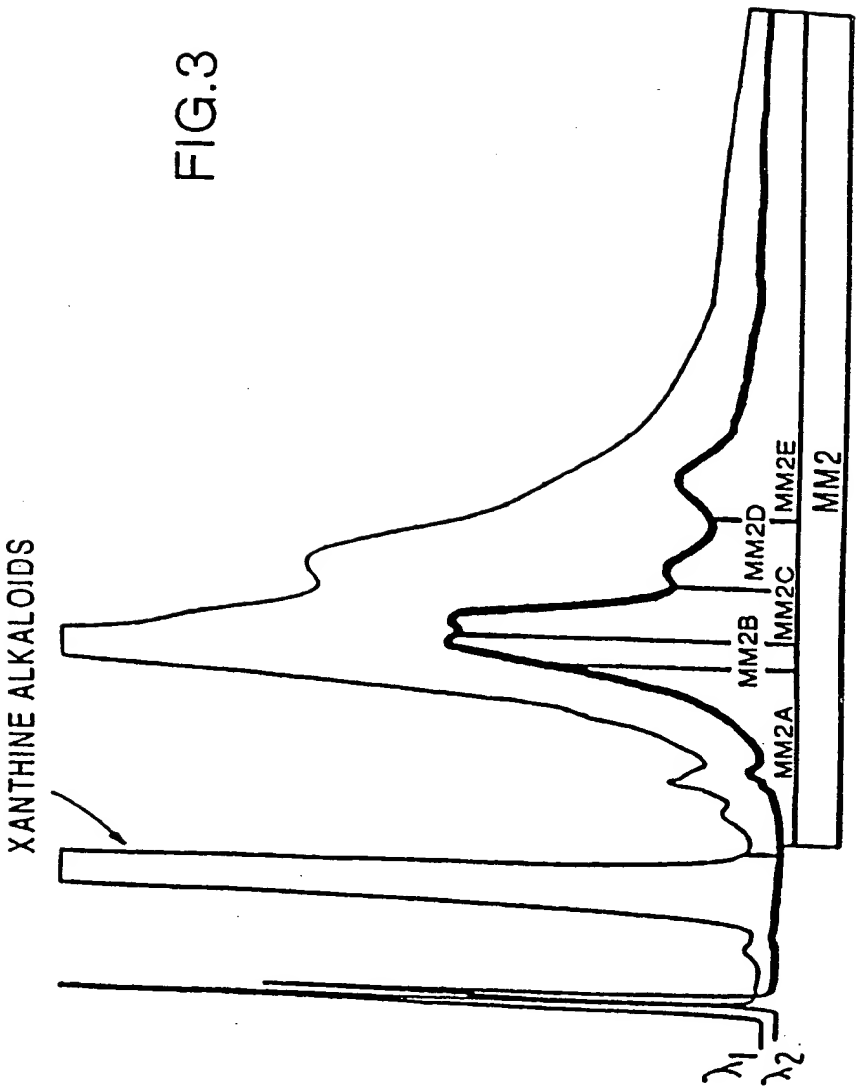


FIG.4

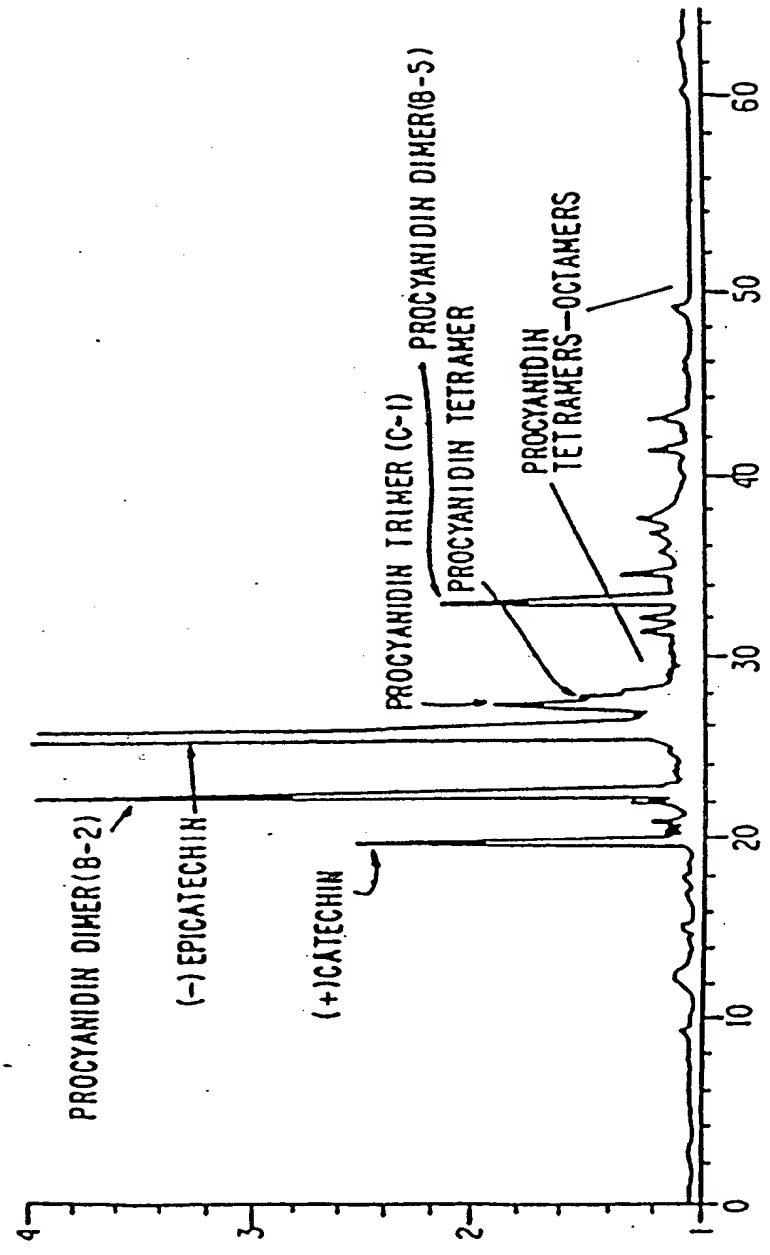

PROCYANIDIN DIMER(B-2)
(-) EPICATECHIN
(+)CATECHIN
PROCYANIDIN TRIMER (C-1)
PROCYANIDIN DIMER(B-5)
PROCYANIDIN TETRAMER
PROCYANIDIN TETRAMERS-OCTAMERS
4
3
2
1
0
10
20
30
40
50
60

FIG.5

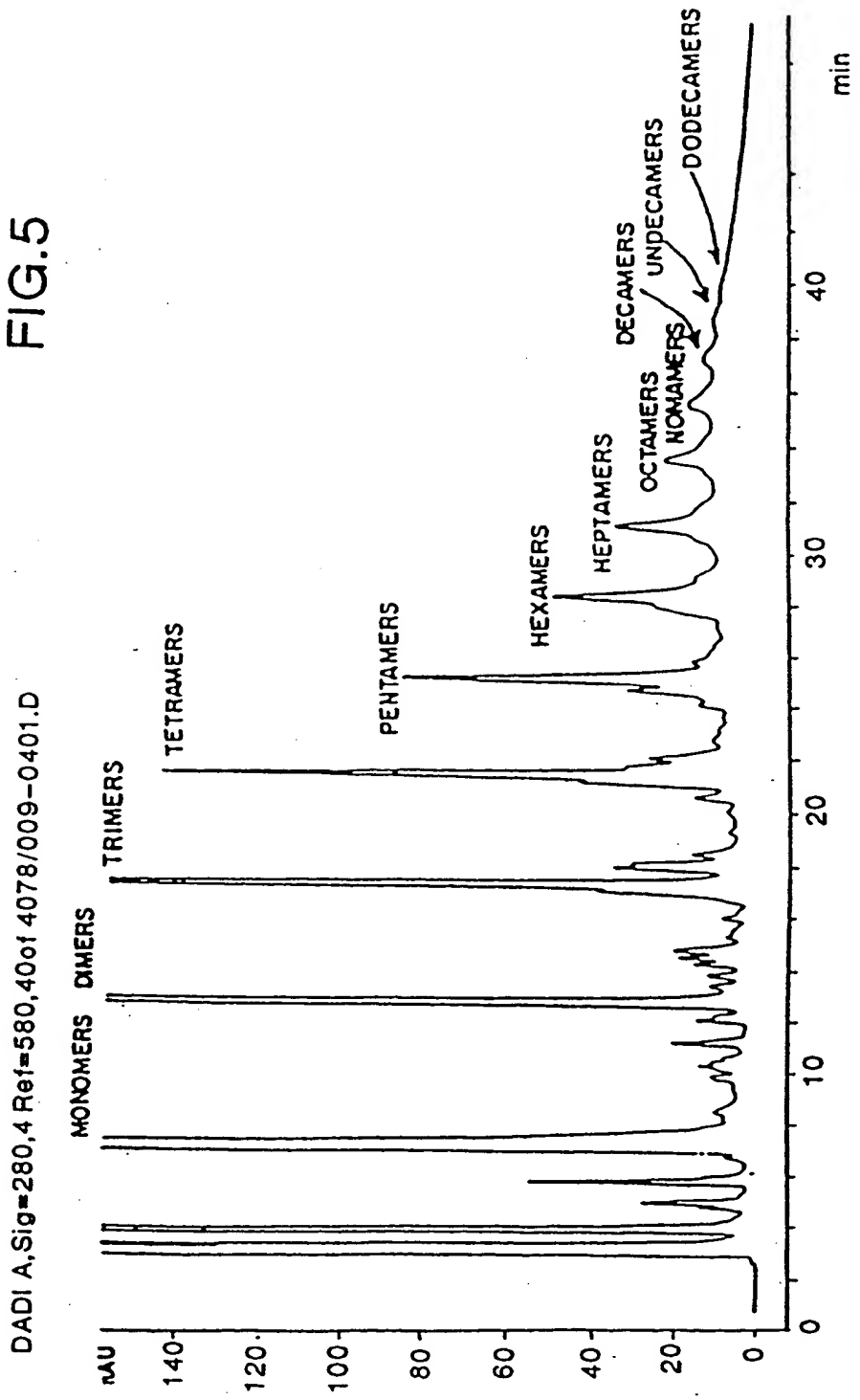

DAD1 A,Sig=280,4 Ref=580,40of 4078/009-0401.D
mAU
140
120
100
80
60
40
20
0
MONOMERS
DIMERS
TRIMERS
TETRAMERS
PENTAMERS
HEXAMERS
HEPTAMERS
OCTAMERS
NONAMERS
DECAMERS
UNDECAMERS
DODECAMERS
0
10
20
30
40
min

FIG.6

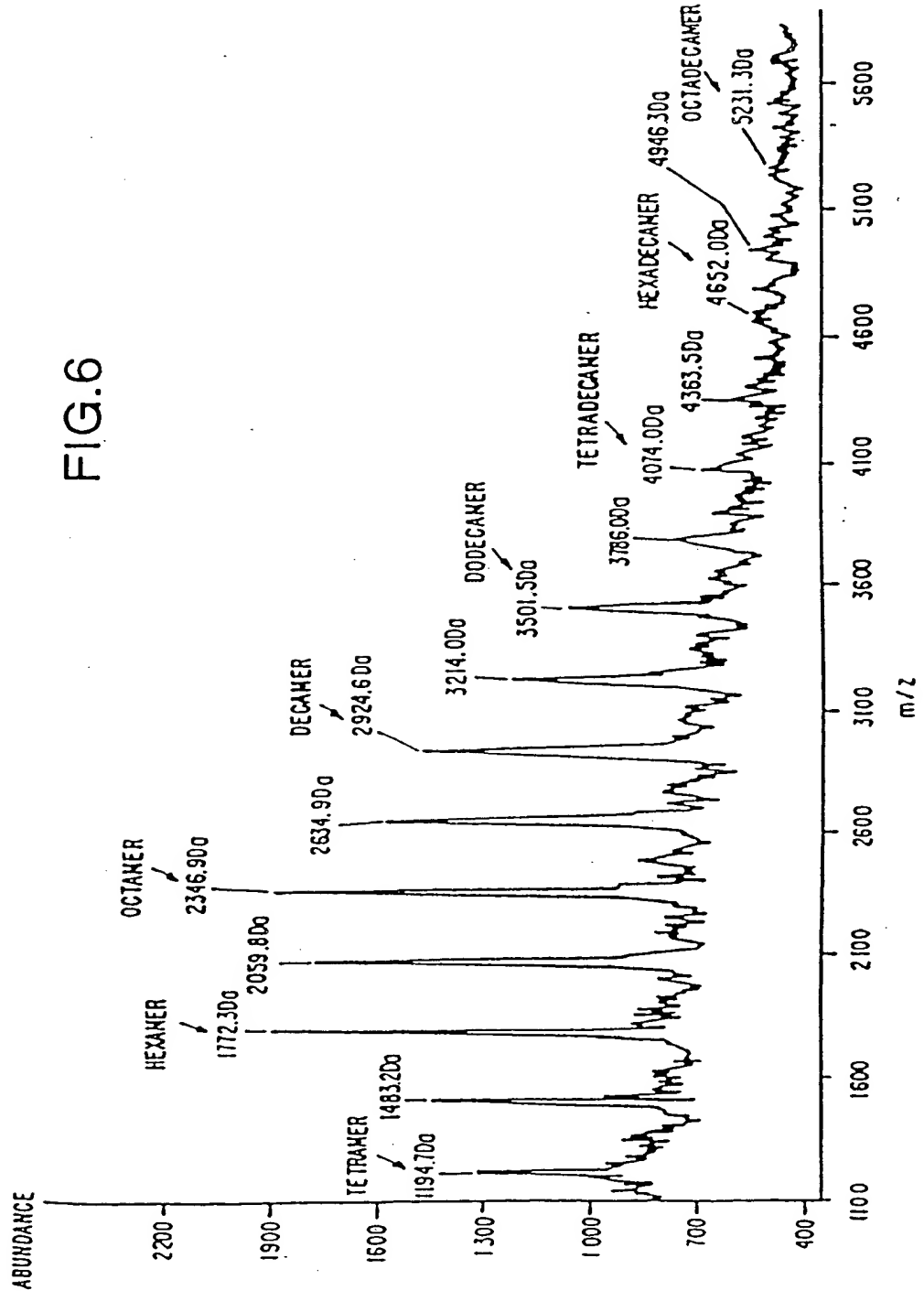

ABUNDANCE
2200
1900
1600
1300
1000
700
400
1100
1600
2100
2600
3100
3600
4100
4600
5100
5600
m/z
TETRAMER
1194.7Da
1483.2Da
HEXAMER
1772.3Da
2059.8Da
OCTAMER
2346.9Da
2634.9Da
DECAMER
2924.6Da
3214.0Da
DODECAMER
3501.5Da
TETRADECAMER
3786.0Da
4074.0Da
HEXADECAMER
4363.5Da
4652.0Da
OCTADECAMER
4946.3Da
5231.3Da

FIG.7

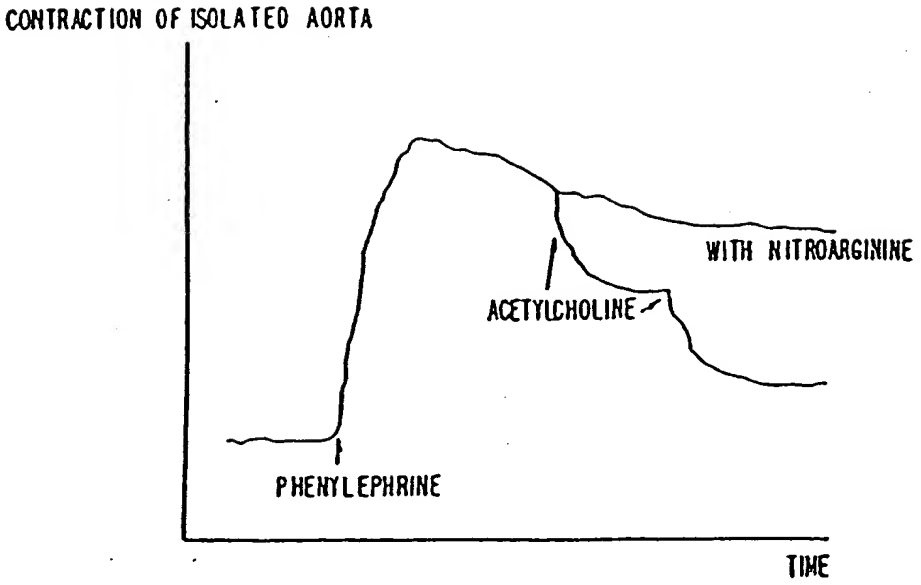
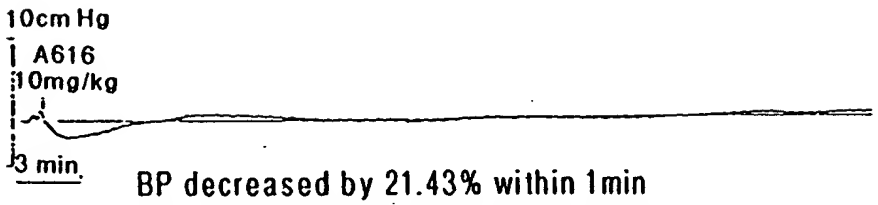

CONTRACTION OF ISOLATED AORTA
WITH NITROARGININE
ACETYLCHOLINE
PHENYLEPHRINE
TIME

FIG.8A

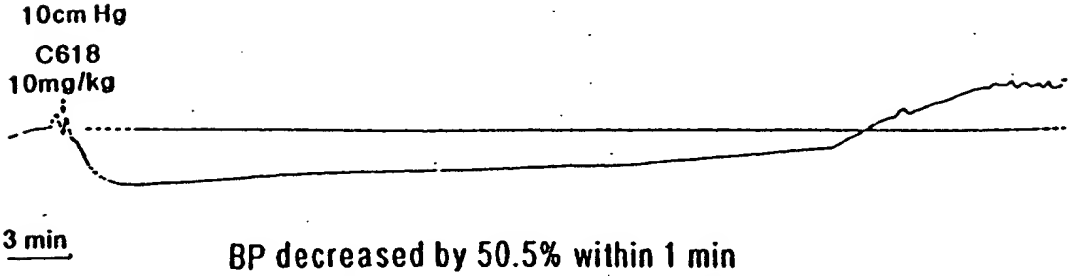
EFFECT OF COCOA PROCYANIDIN FRACTION A ON BLOOD PRESSURE



BP decreased by 21.43% within 1min
BP back to normal value after 1.5 min

FIG.8B

EFFECT OF COCOA PROCYANIDIN FRACTION C ON BLOOD PRESSURE



BP decreased by 50.5% within 1 min
BP back to normal value after 5 min

EFFECT OF COCOA PROCYANIDIN FRACTIONS ON ARTERIAL BLOOD PRESSURE IN ANESTHISIZED GUINEA PIGS

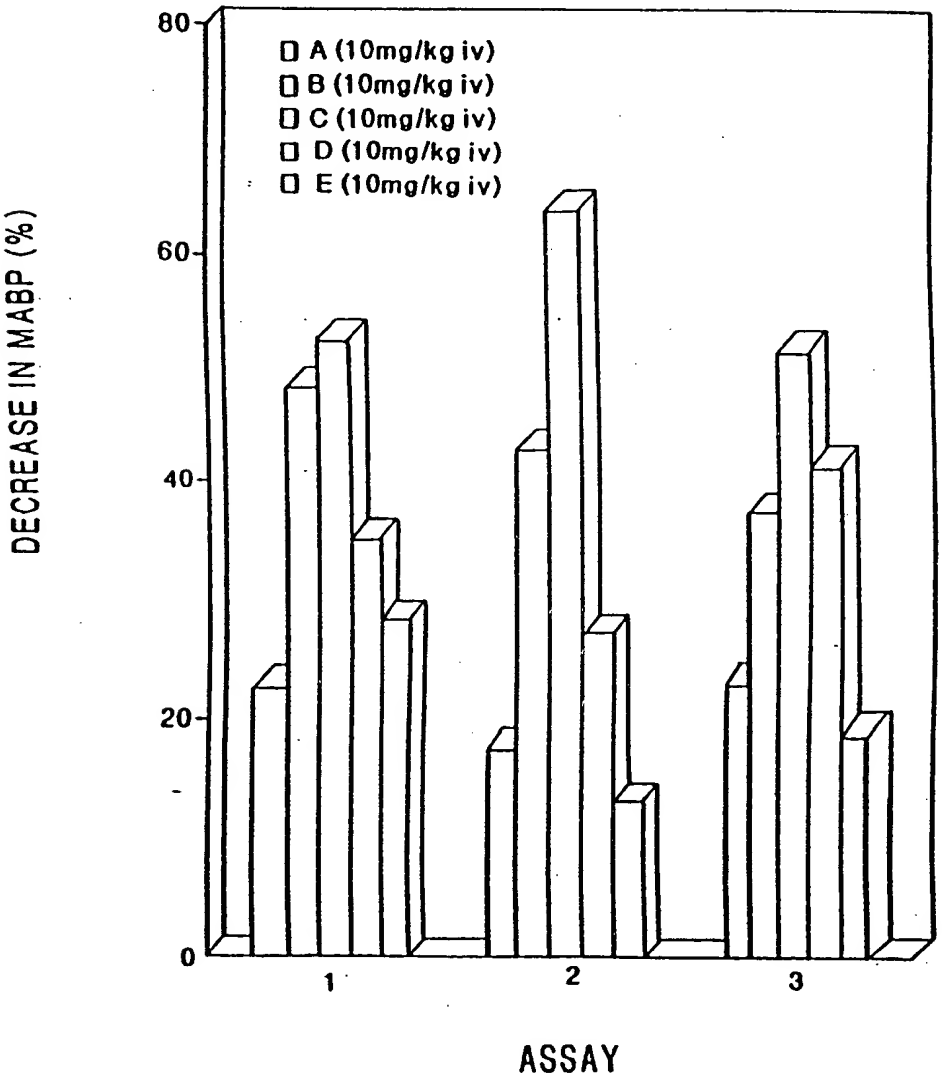


FIG.9

EFFECT OF L-NMMA ON THE ALTERATIONS OF ARTERIAL BLOOD PRESSURE IN ANESTHISIZED GUINEA PIGS INDUCED BY COCOA PROCYANIDIN FRACTION C

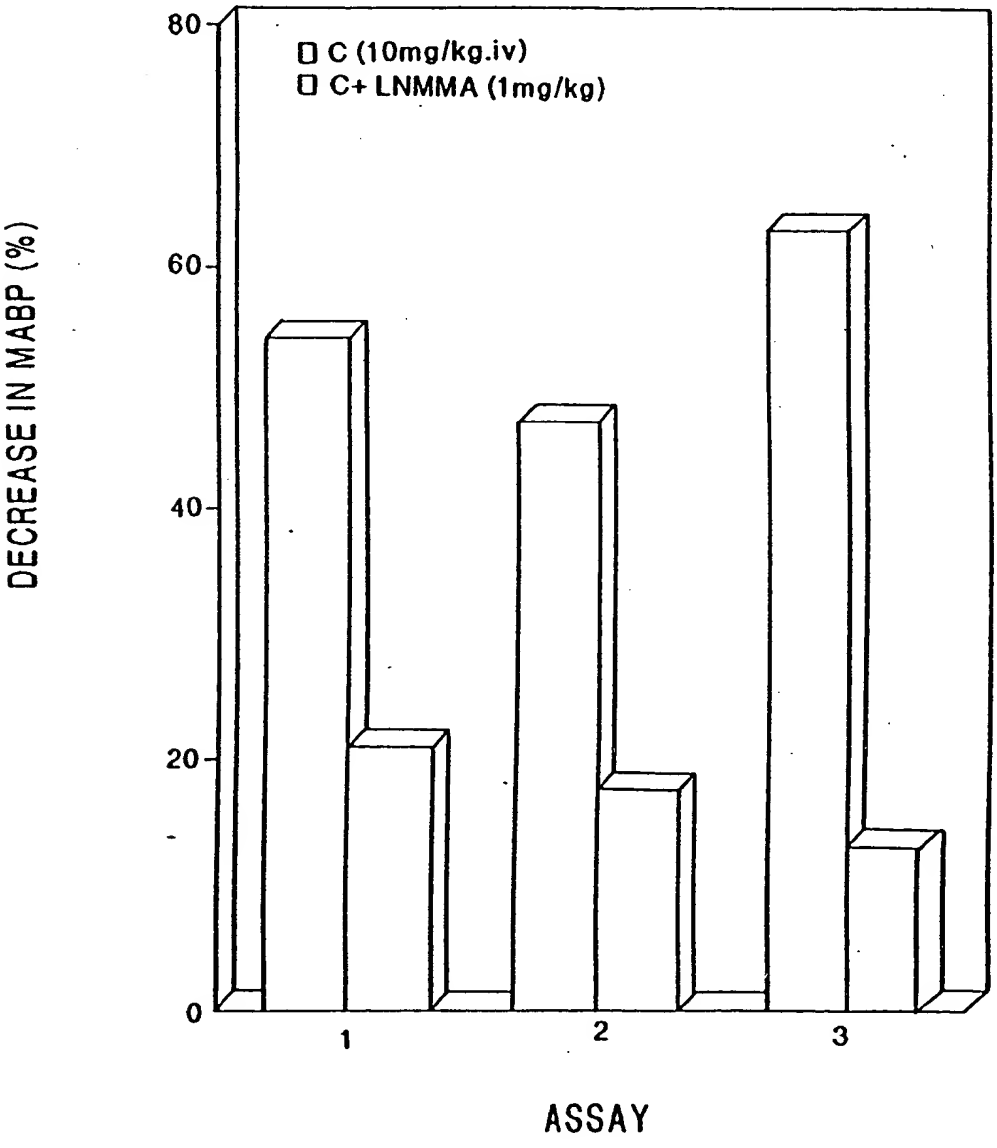


FIG.10

EFFECT OF BRADYKININ ON NO PRODUCTION BY HUVEC

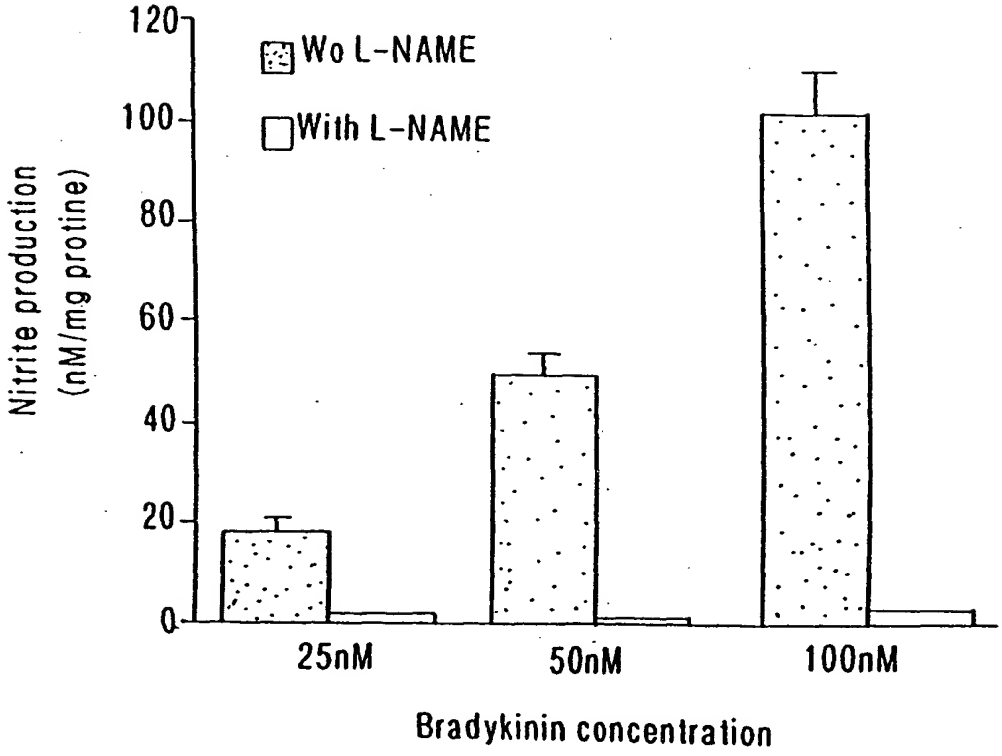


FIG.11

EFFECT OF COCOA PROCYANIDIN FRACTIONS ON NO PRODUCTION BY HUVEC

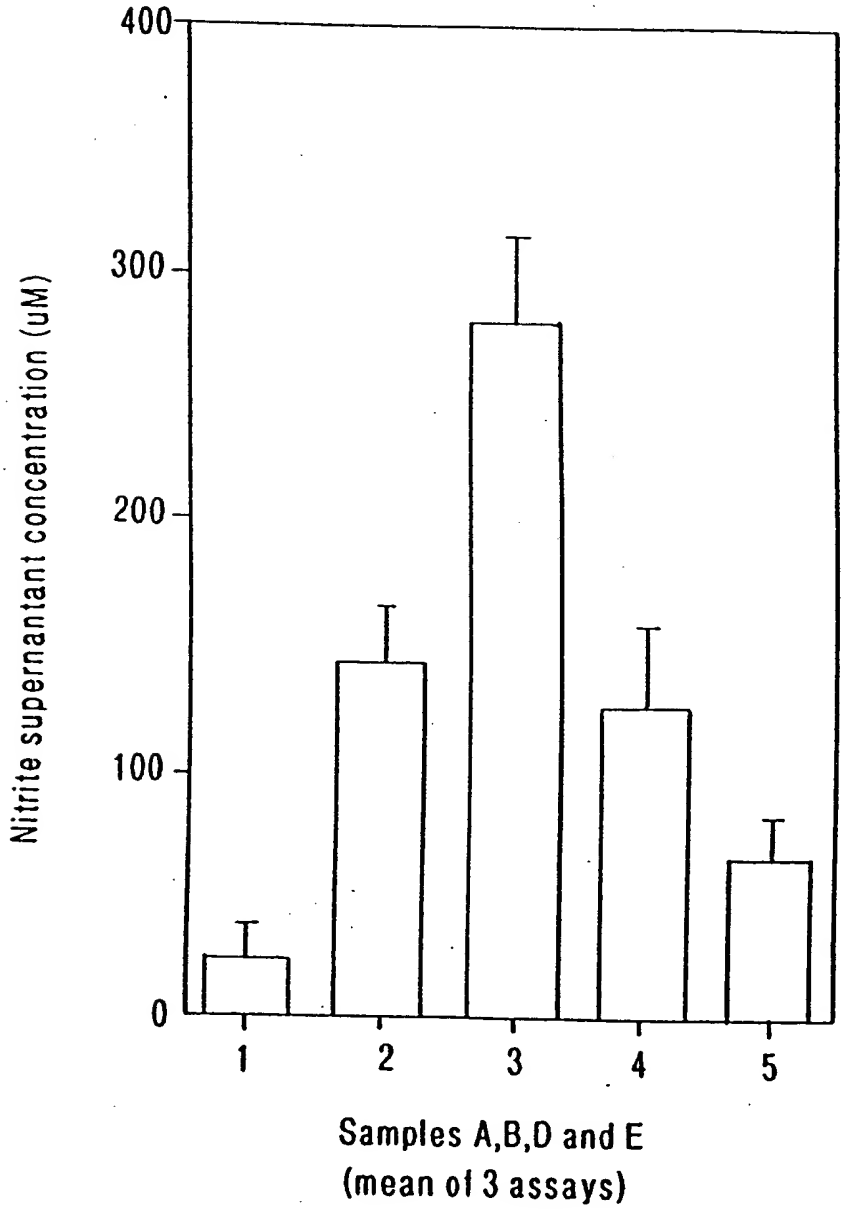


FIG.12

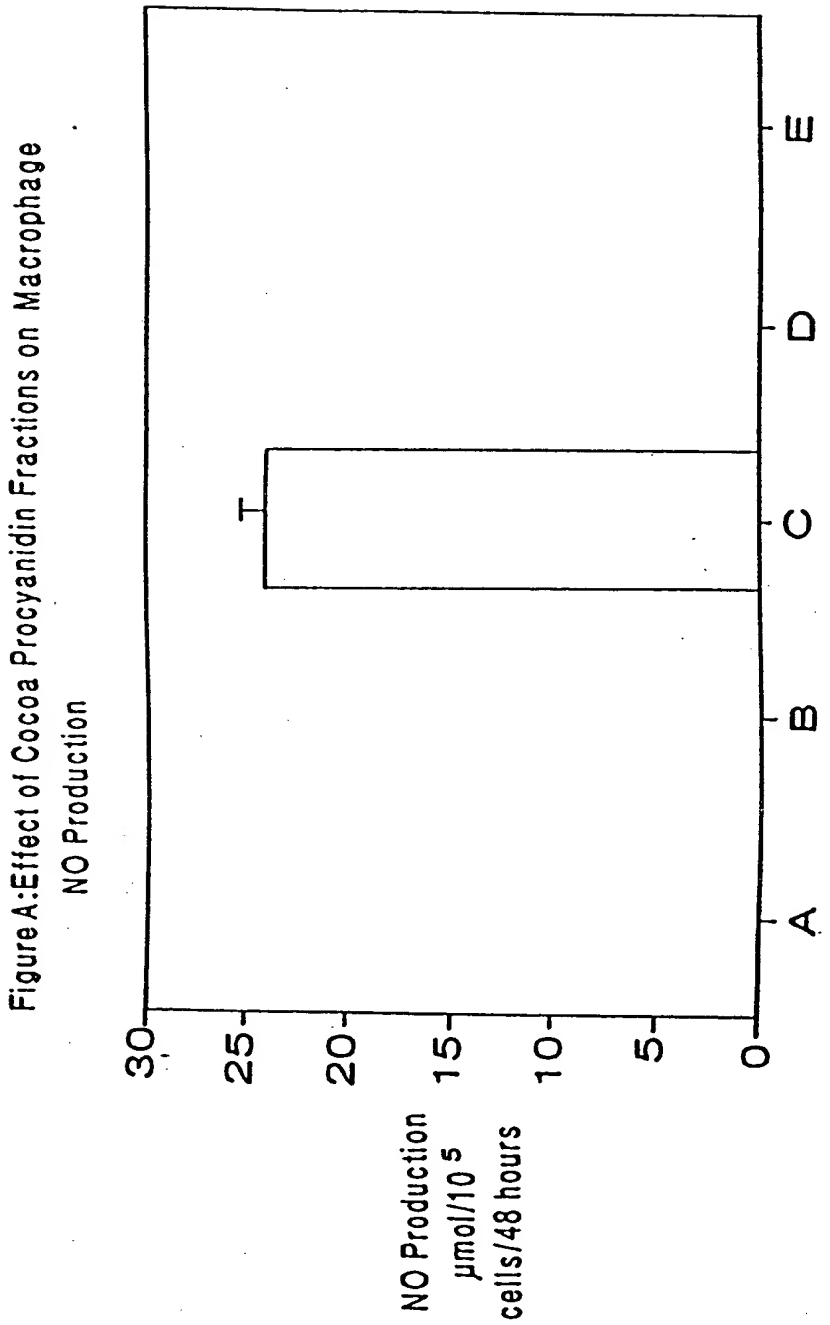


Figure A:Effect of Cocoa Procyanidin Fractions on Macrophage NO Production
NO Production µmol/10 5 cells/48 hours
30
25
20
15
10
5
0
A
B
C
D
E
Cocoa Procyanidin Fractions

FIG.13

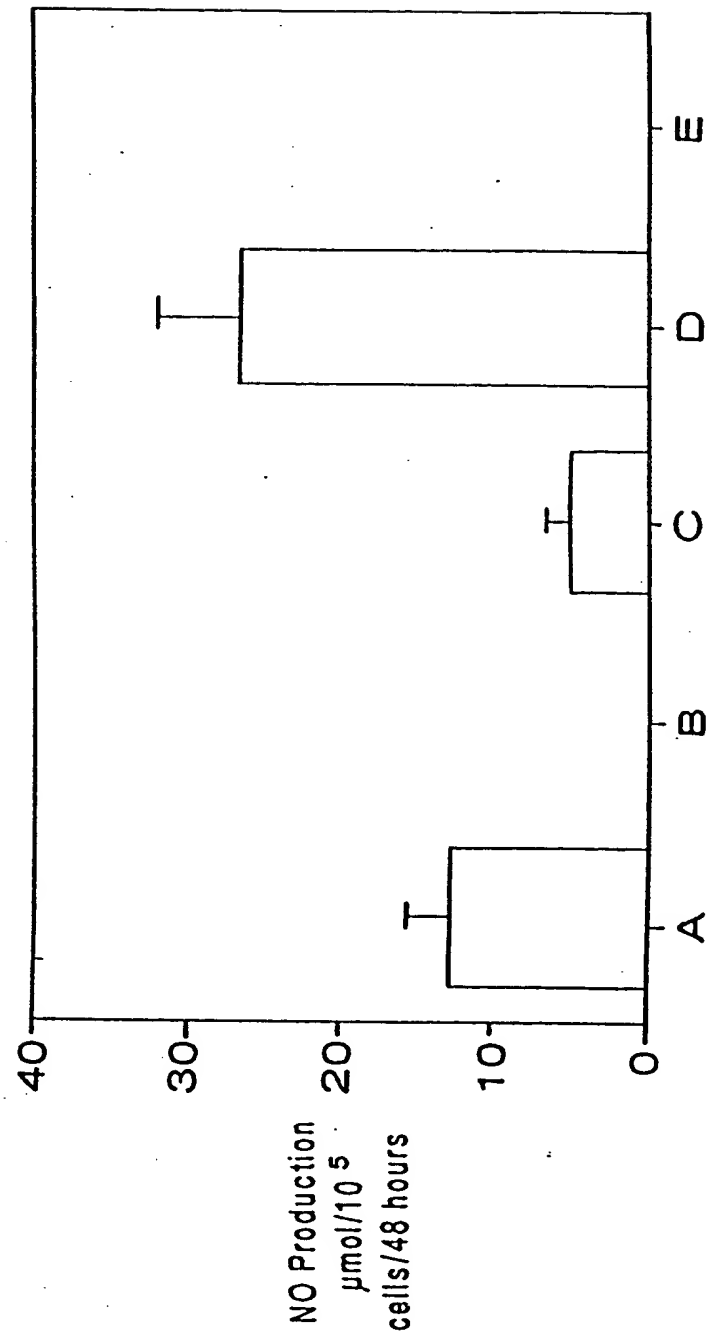


Figure B:Effect of Cocoa Procyanidin Fractions on LPS Induced and γ-Interferon Primed Macrophages
NO Production µmol/10 5 cells/48 hours
40
30
20
10
0
A
B
C
D
E
Cocoa Procyanidin Fractions

FIG.14

FIG.15A

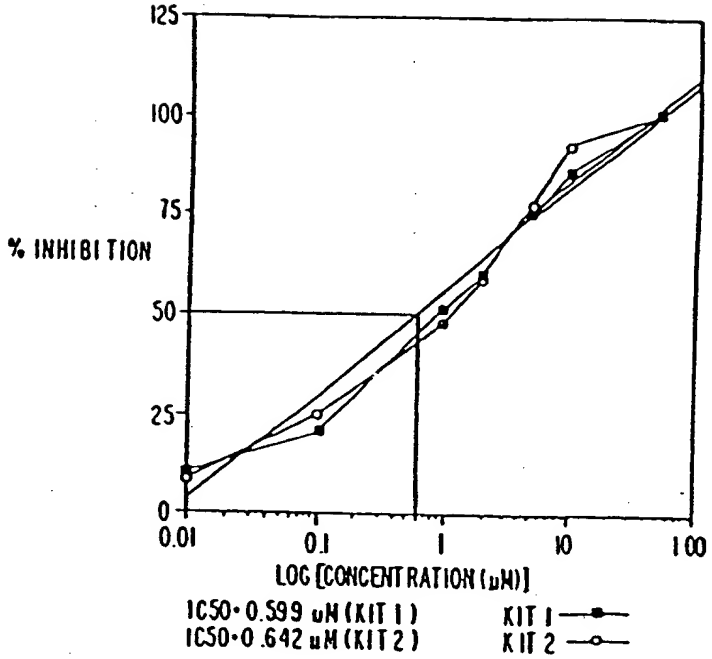


FIG.15B

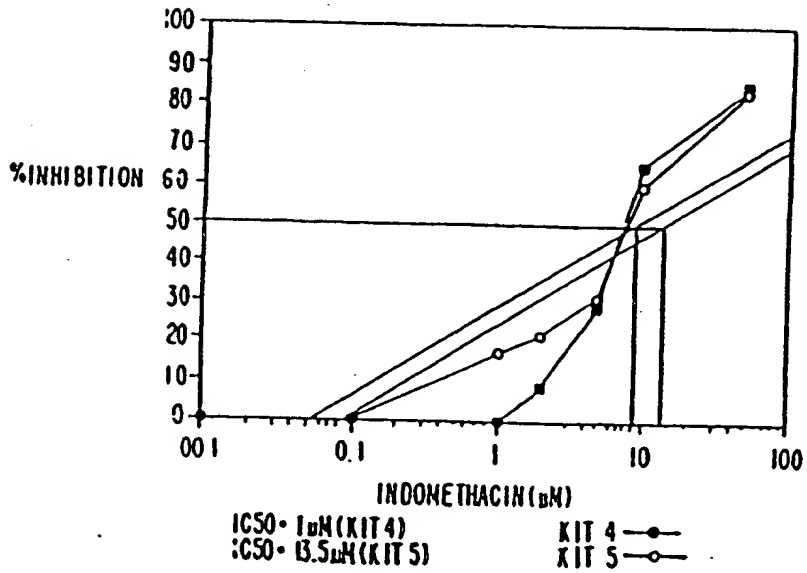
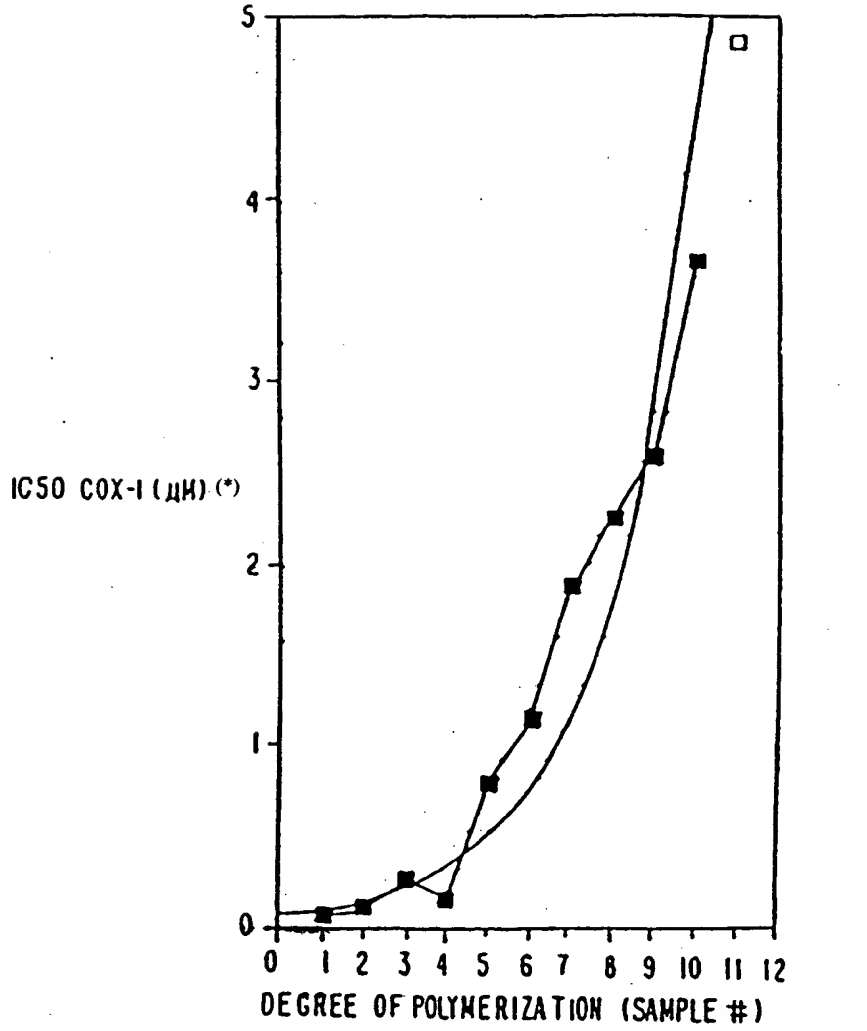
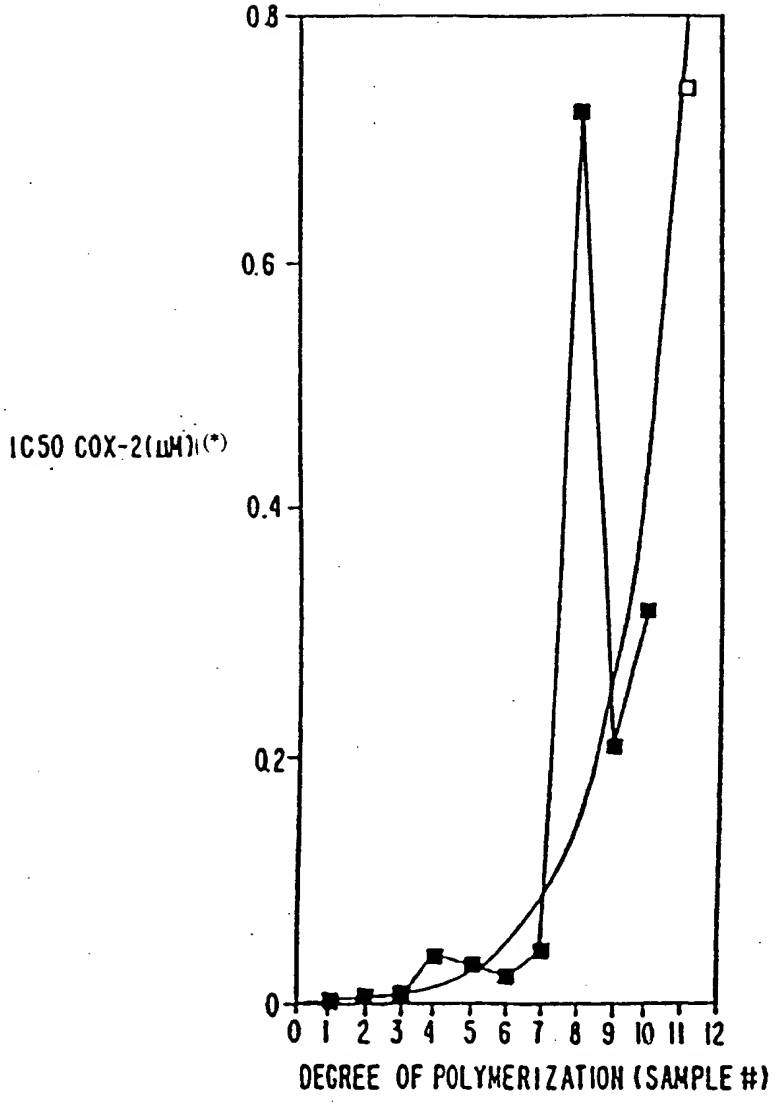


FIG.16A



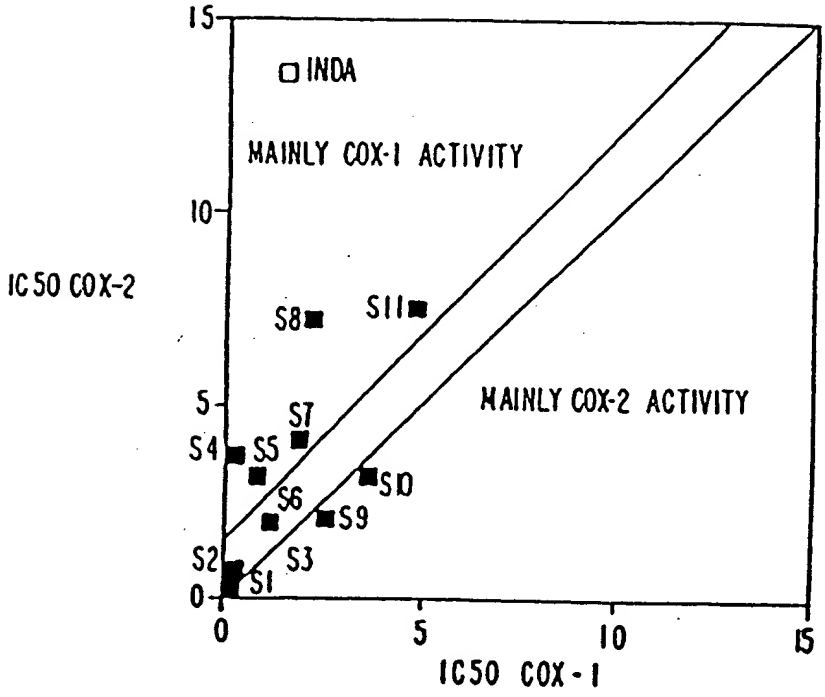
(*) WITH THE EXCEPTION OF SAMPLE S11 EXPRESSED AS mg/ml

FIG.16B



(*) WITH THE EXCEPTION OF SAMPLE S11 EXPRESSED AS mg/ml

FIG.17



(*) WITH THE EXEPTION OF SAMPLE S11

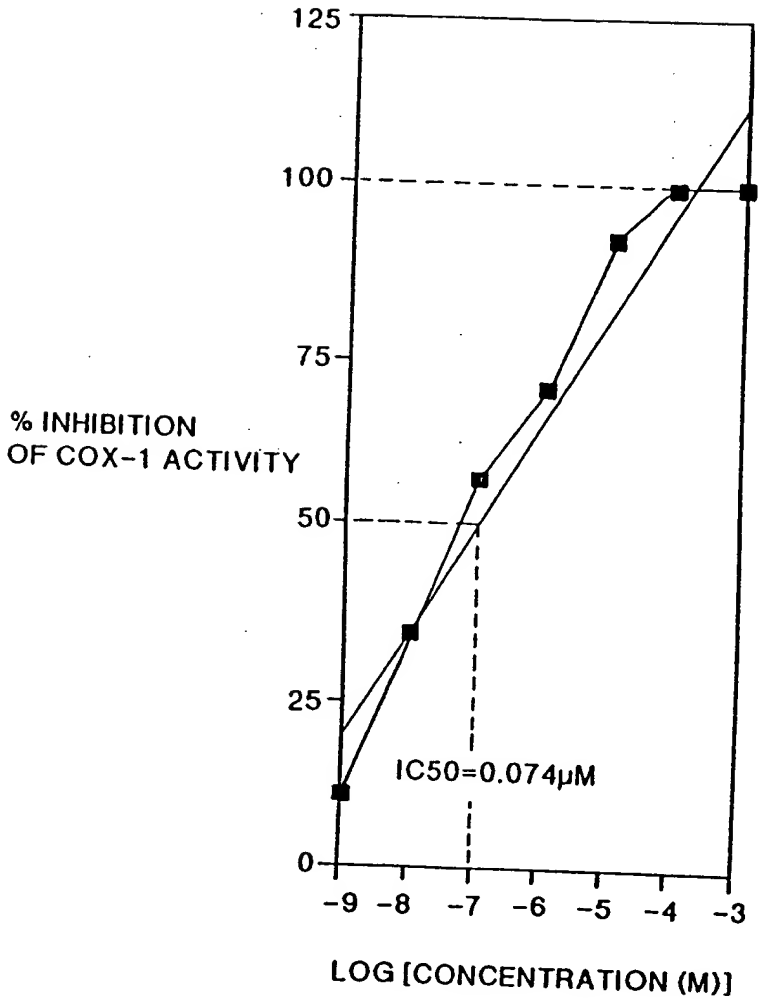

125
100
75
50
25
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=0.074μM
-9 -8 -7 -6 -5 -4 -3
LOG [CONCENTRATION (M)]

FIG.18A

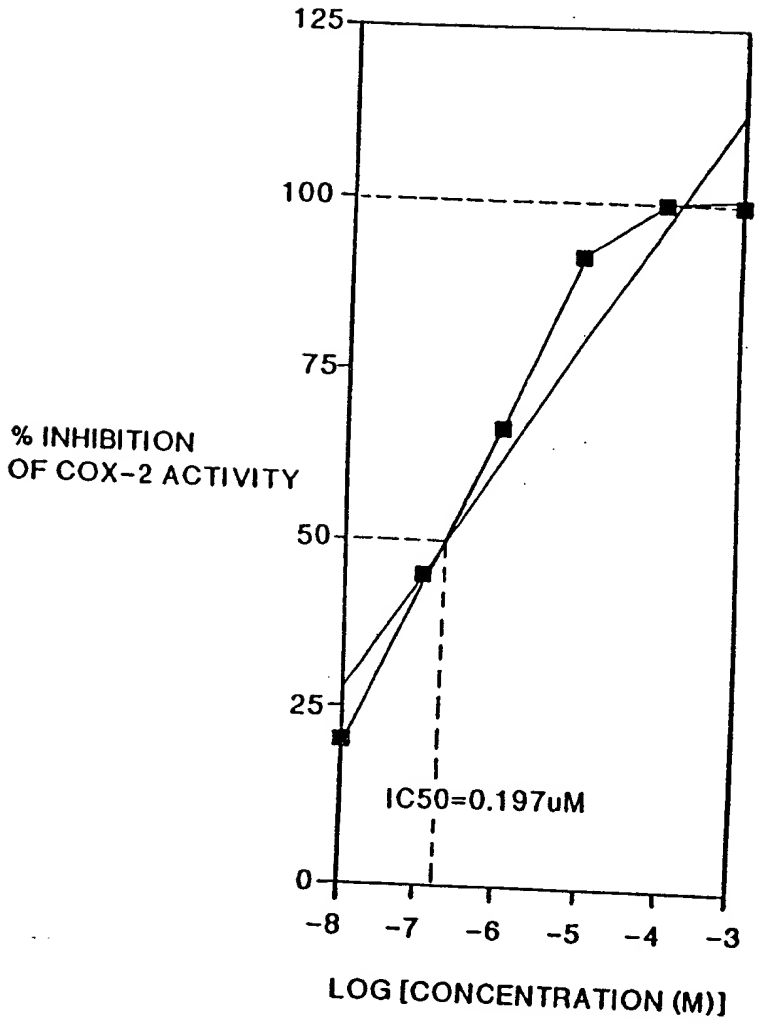

125
100
75
50
25
0
% INHIBITION
OF COX-2 ACTIVITY
IC50=0.197uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18B

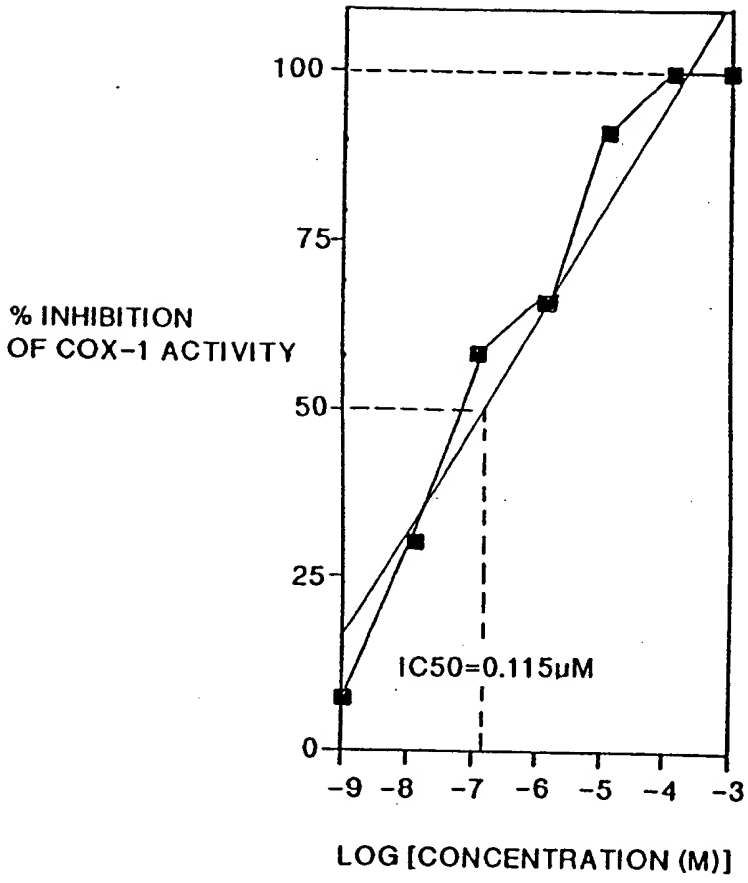

100
75
50
25
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=0.115μM
-9 -8 -7 -6 -5 -4 -3
LOG [CONCENTRATION (M)]

FIG.18C

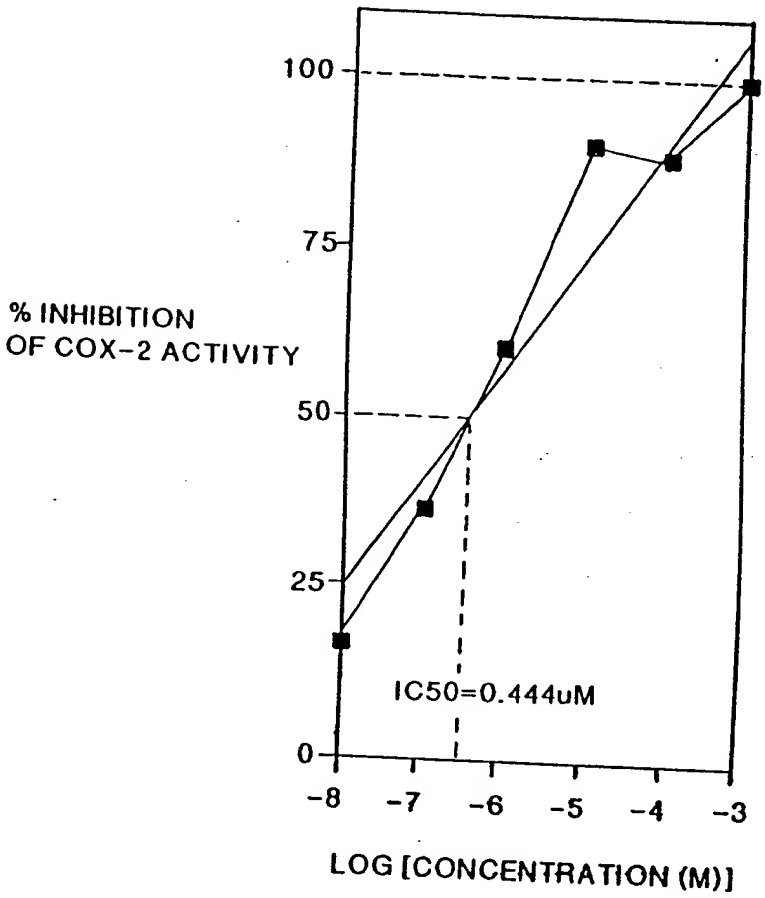

100
75
50
25
0
% INHIBITION
OF COX-2 ACTIVITY
IC50=0.444uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18D

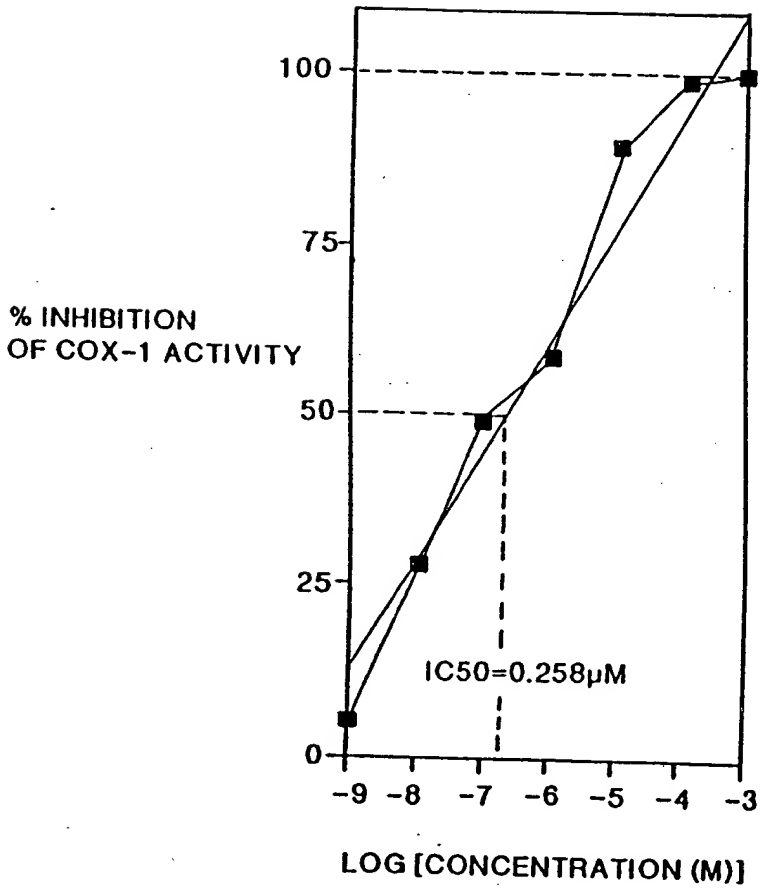

100
75
50
25
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=0.258μM
-9 -8 -7 -6 -5 -4 -3
LOG [CONCENTRATION (M)]

FIG.18E

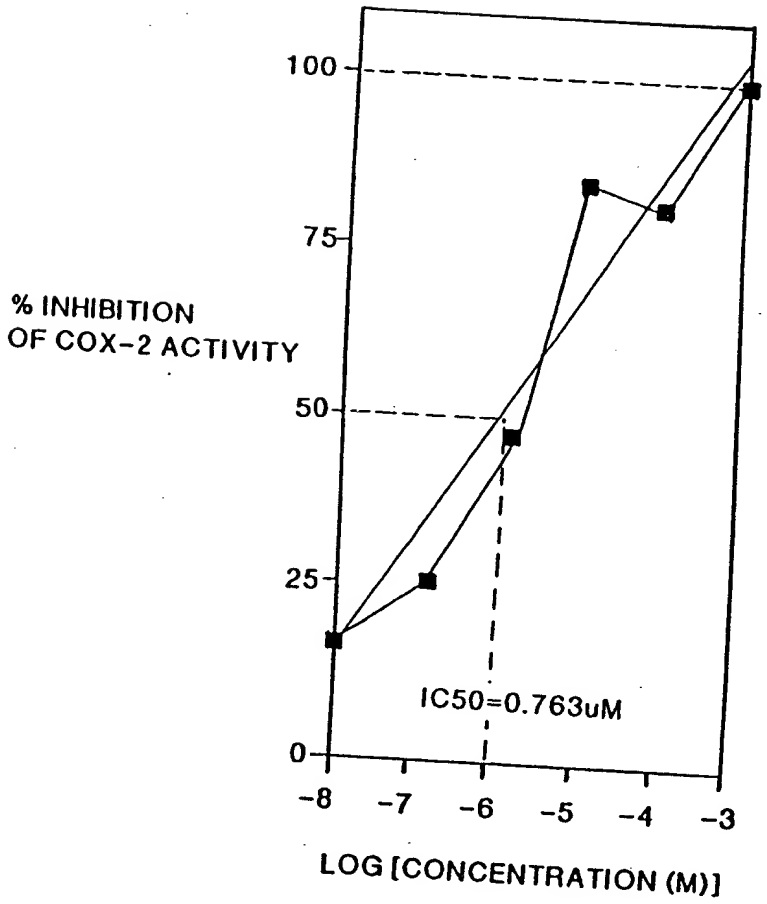

100
75
50
25
0
% INHIBITION
OF COX-2 ACTIVITY
IC50=0.763uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18F

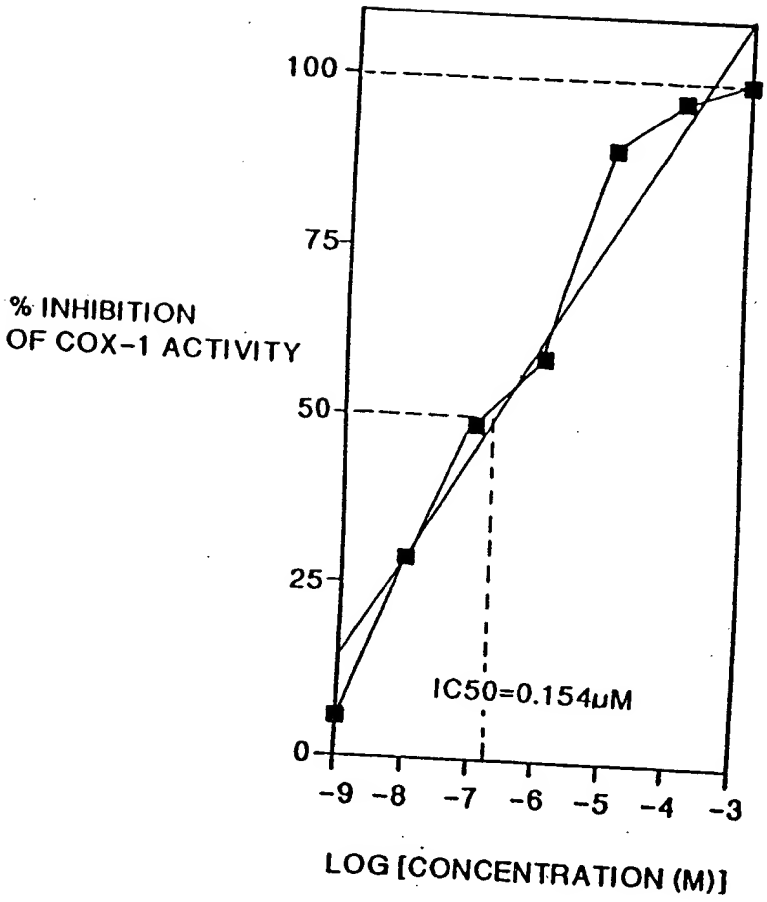

100
75
50
25
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=0.154μM
-9 -8 -7 -6 -5 -4 -3
LOG [CONCENTRATION (M)]

FIG.18G

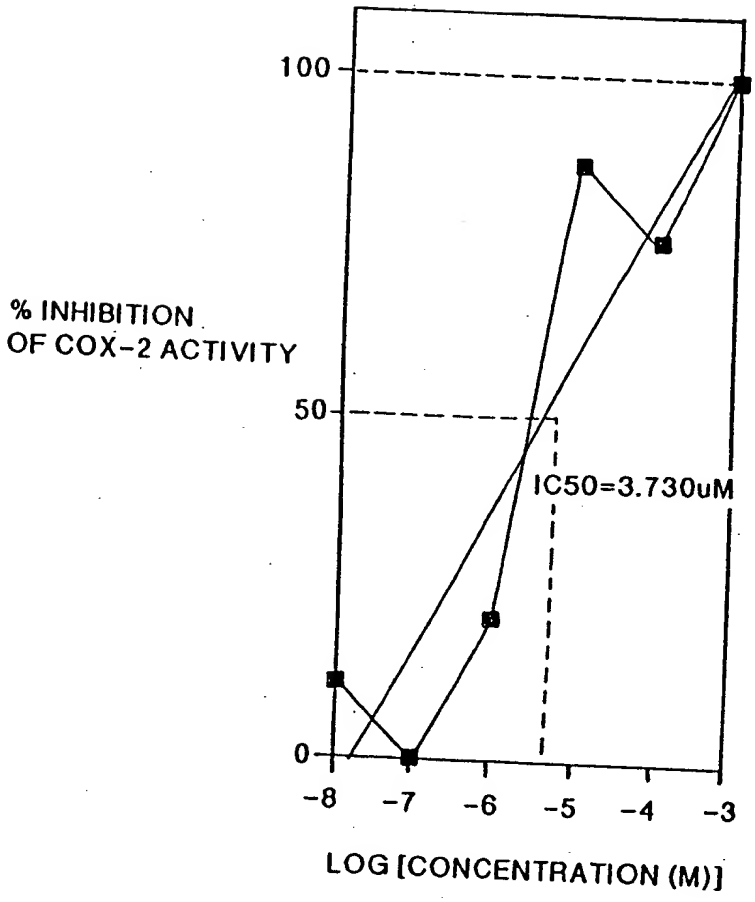

100
50
0
% INHIBITION
OF COX-2 ACTIVITY
IC50=3.730uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18H

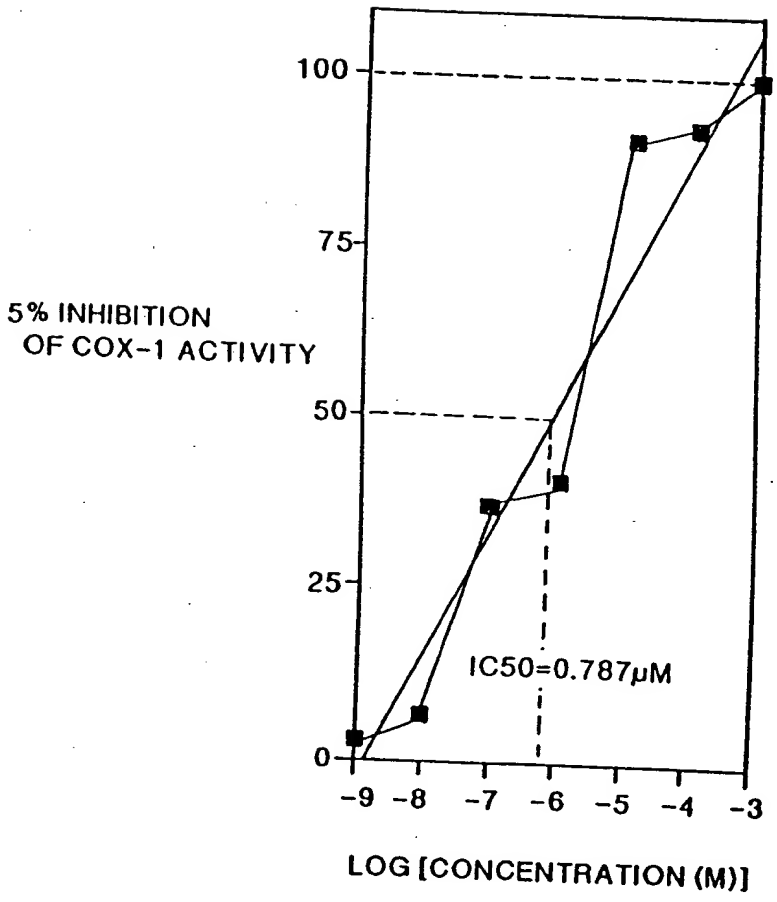

100
75
50
25
0
5% INHIBITION
OF COX-1 ACTIVITY
IC50=0.787μM
-9 -8 -7 -6 -5 -4 -3
LOG [CONCENTRATION (M)]

FIG.18 I

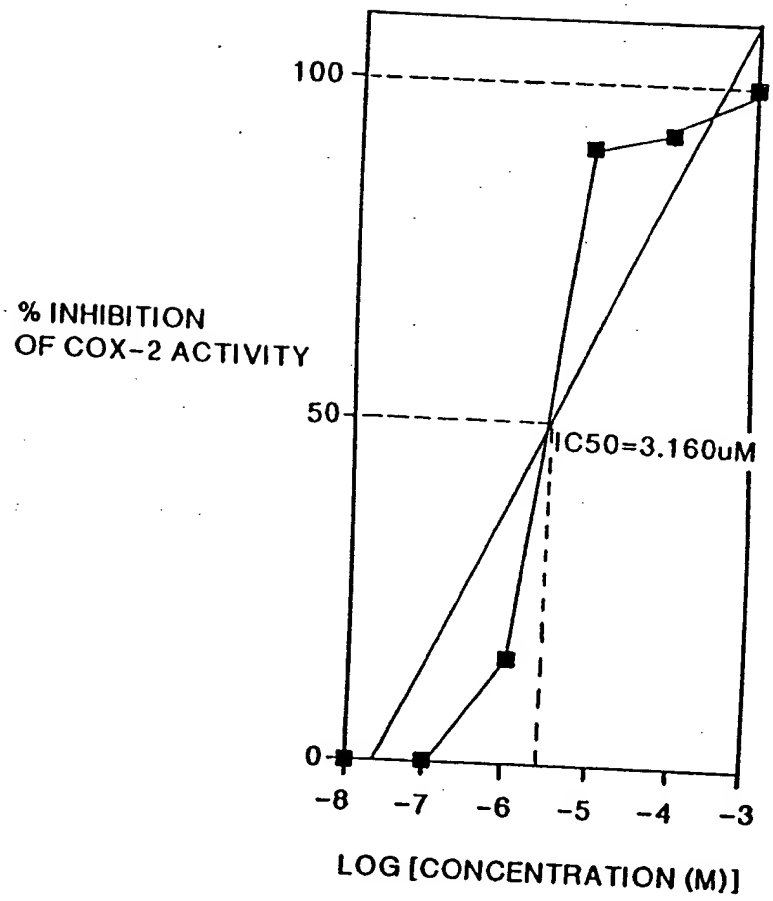

100
50
0
% INHIBITION
OF COX-2 ACTIVITY
IC50=3.160uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18J

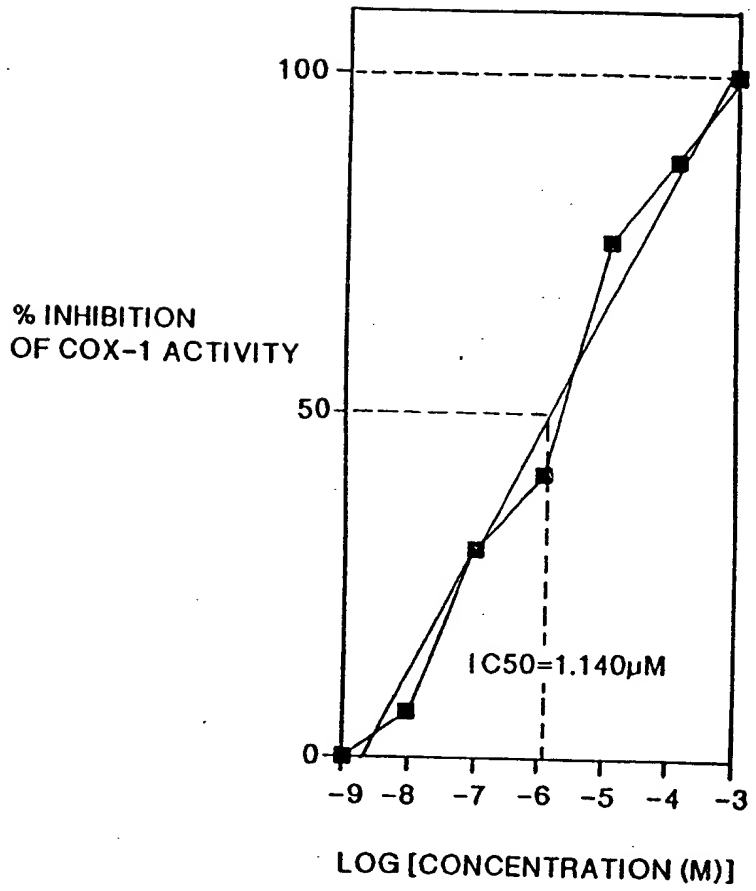

100
50
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=1.140μM
-9 -8 -7 -6 -5 -4 -3
LOG [CONCENTRATION (M)]

FIG.18K

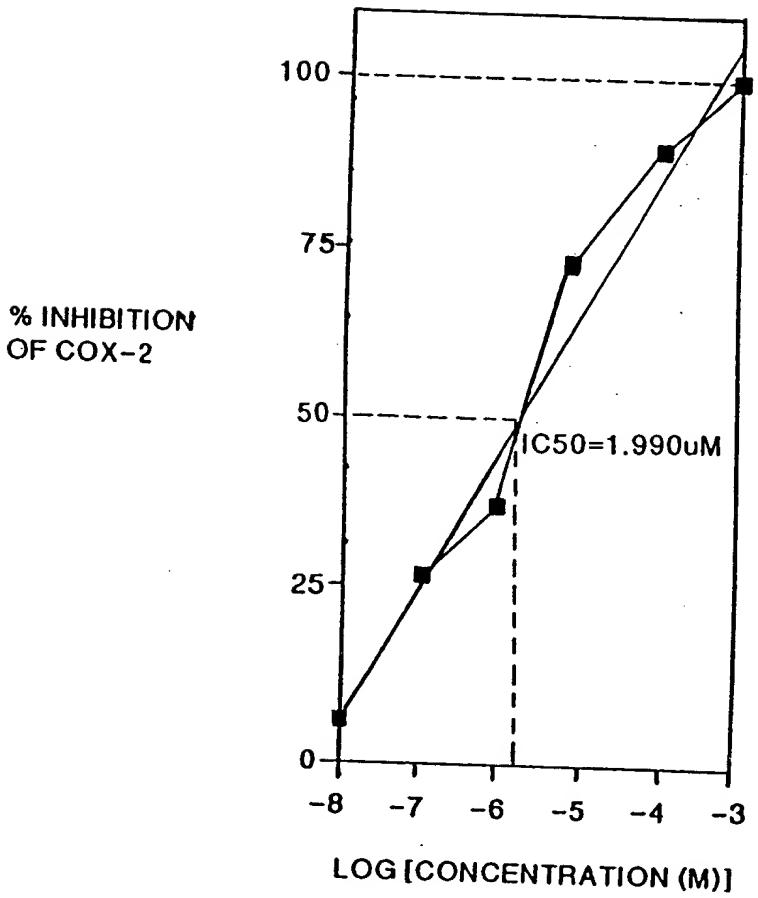

% INHIBITION
OF COX-2
100
75
50
25
0
IC50=1.990uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18L

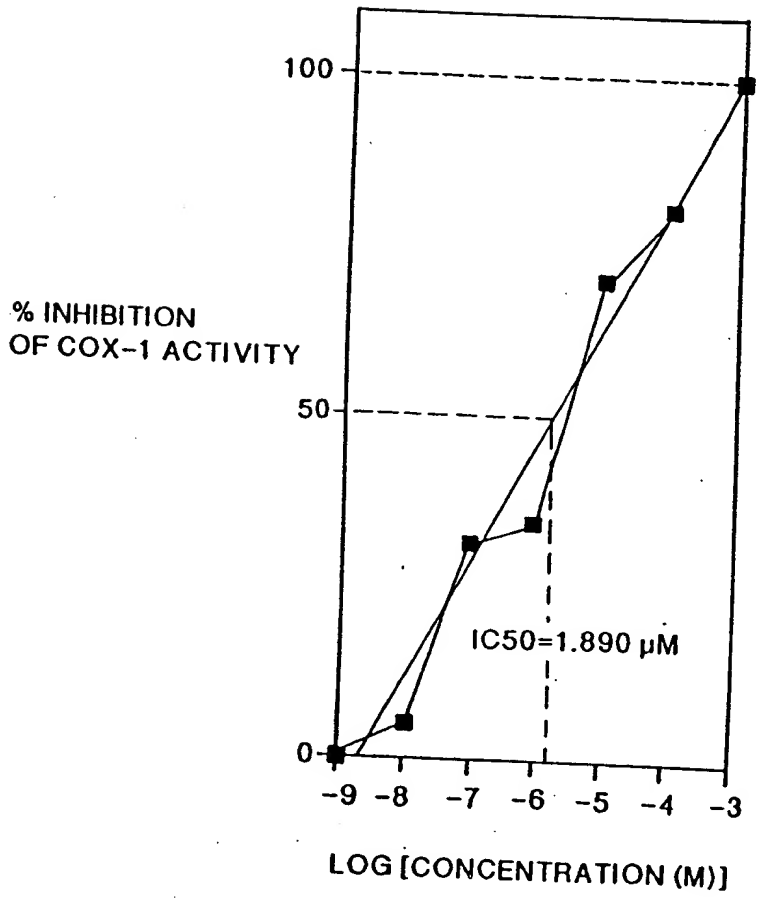

100
50
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=1.890 µM
-9 -8 -7 -6 -5 -4 -3
LOG [CONCENTRATION (M)]

FIG.18M

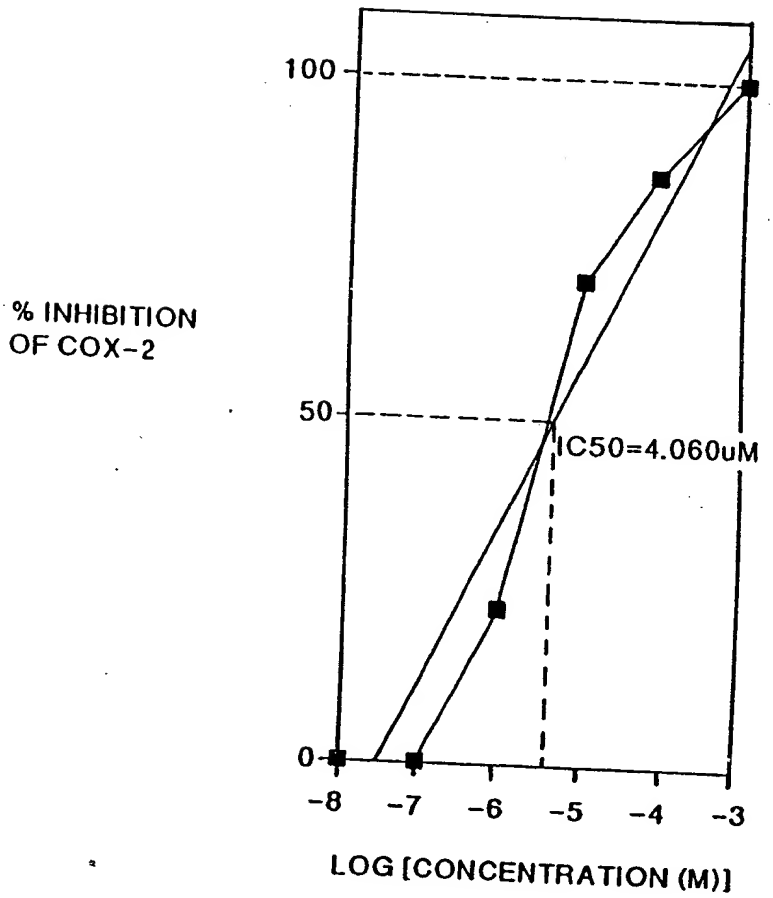

% INHIBITION
OF COX-2
100
50
0
IC50=4.060uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18N

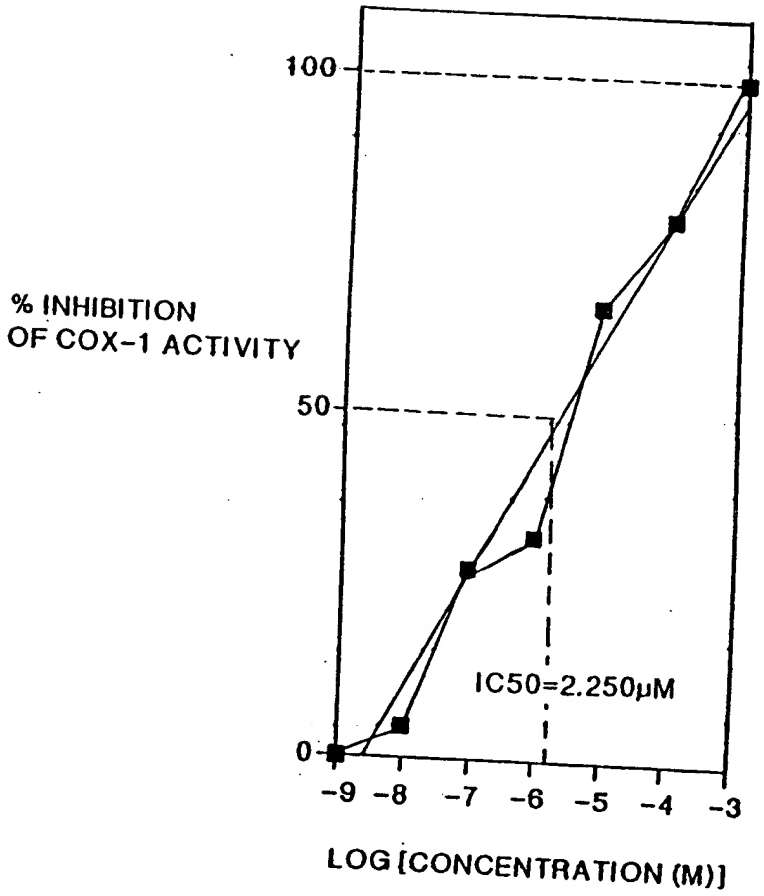

100
50
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=2.250μM
-9 -8 -7 -6 -5 -4 -3
LOG [CONCENTRATION (M)]

FIG.18O

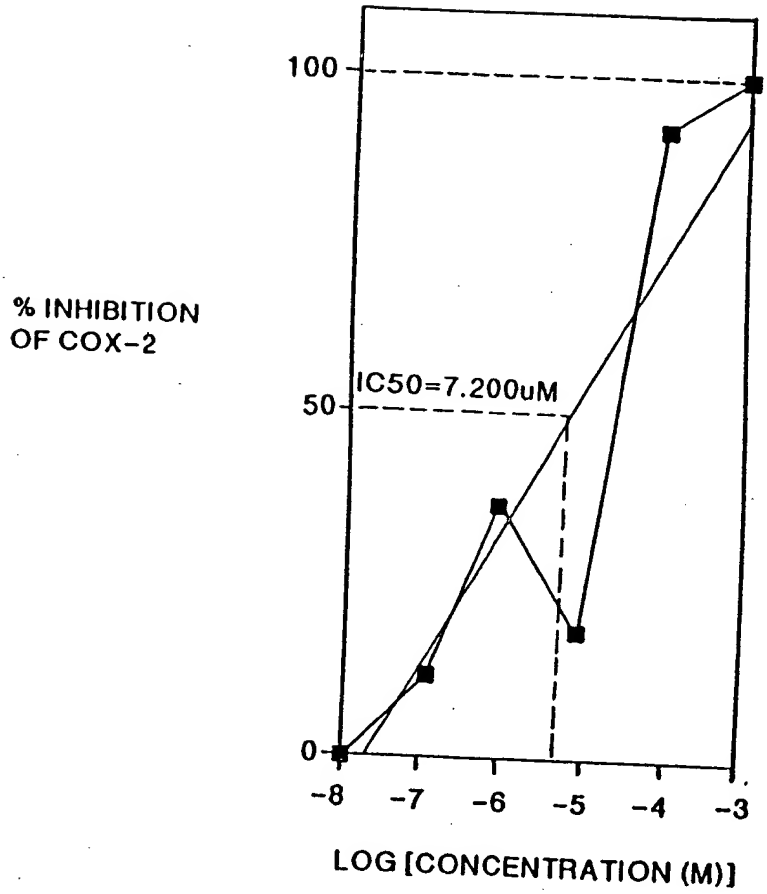

% INHIBITION
OF COX-2
100
50
0
IC50=7.200uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18P

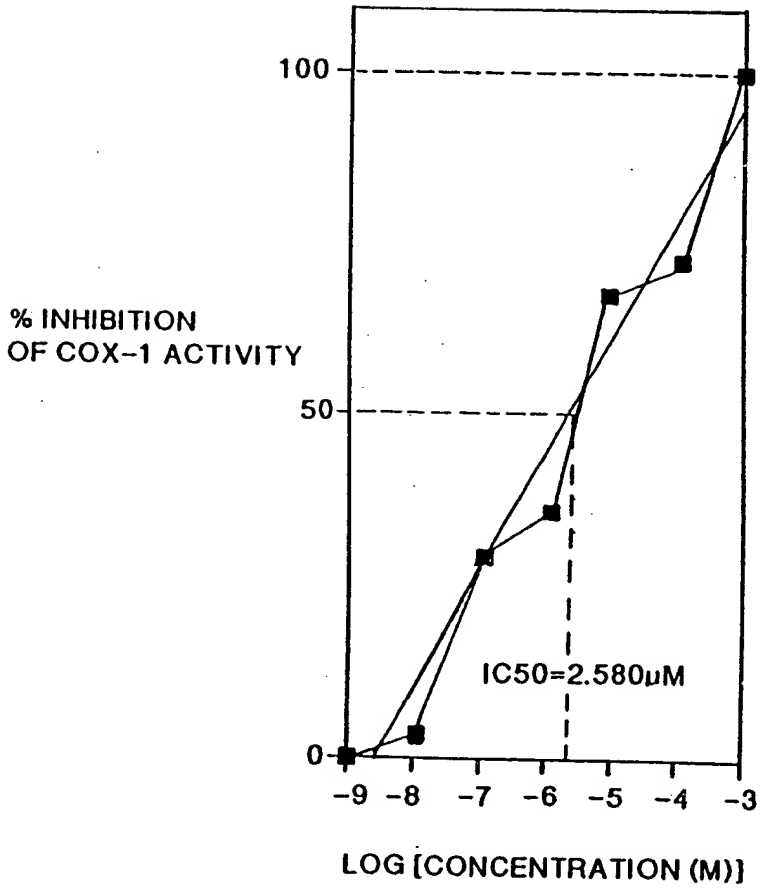

100
50
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=2.580μM
-9 -8 -7 -6 -5 -4 -3
LOG [CONCENTRATION (M)]

FIG.18Q

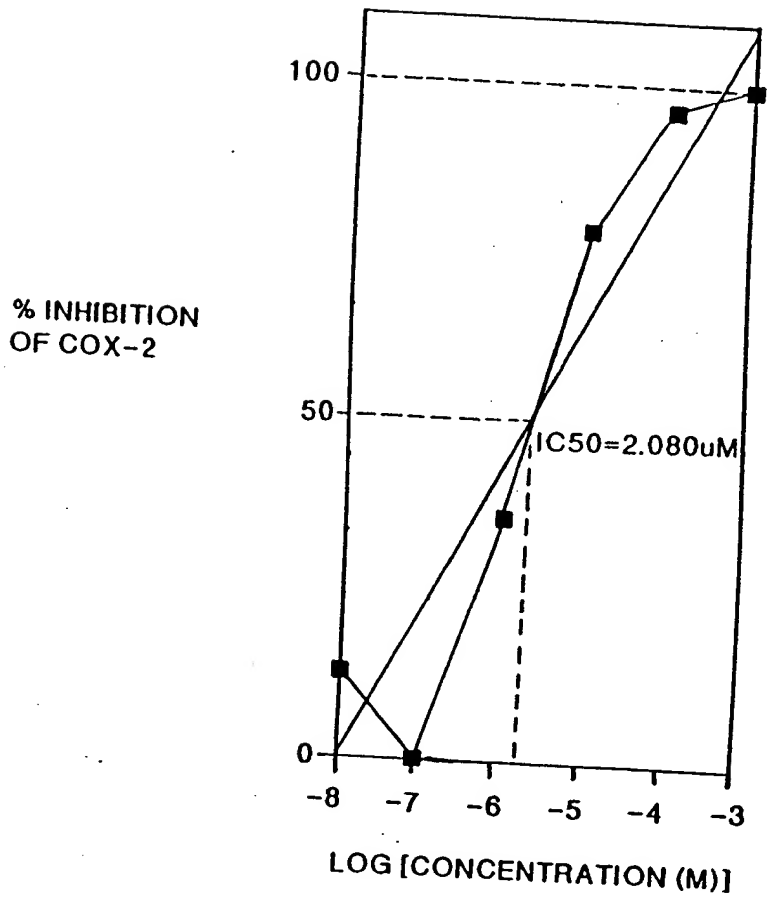

% INHIBITION
OF COX-2
100
50
0
IC50=2.080uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18R

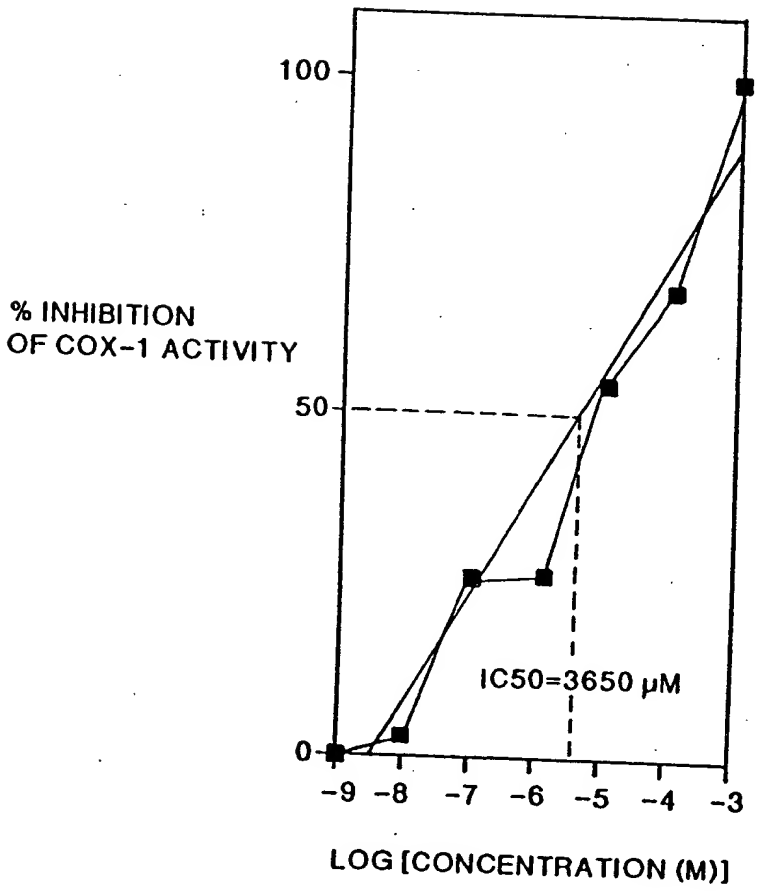

100
50
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=3650 μM
-9 -8 -7 -6 -5 -4 -3
LOG [CONCENTRATION (M)]

FIG.18S

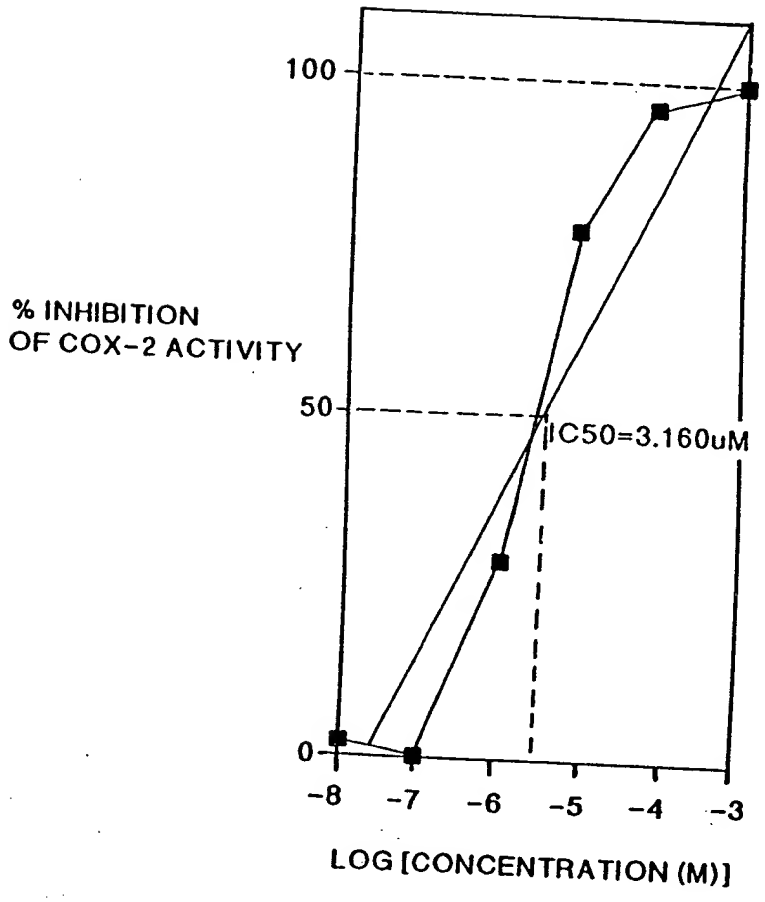

100
50
0
% INHIBITION
OF COX-2 ACTIVITY
IC50=3.160uM
-8
-7
-6
-5
-4
-3
LOG [CONCENTRATION (M)]

FIG.18T

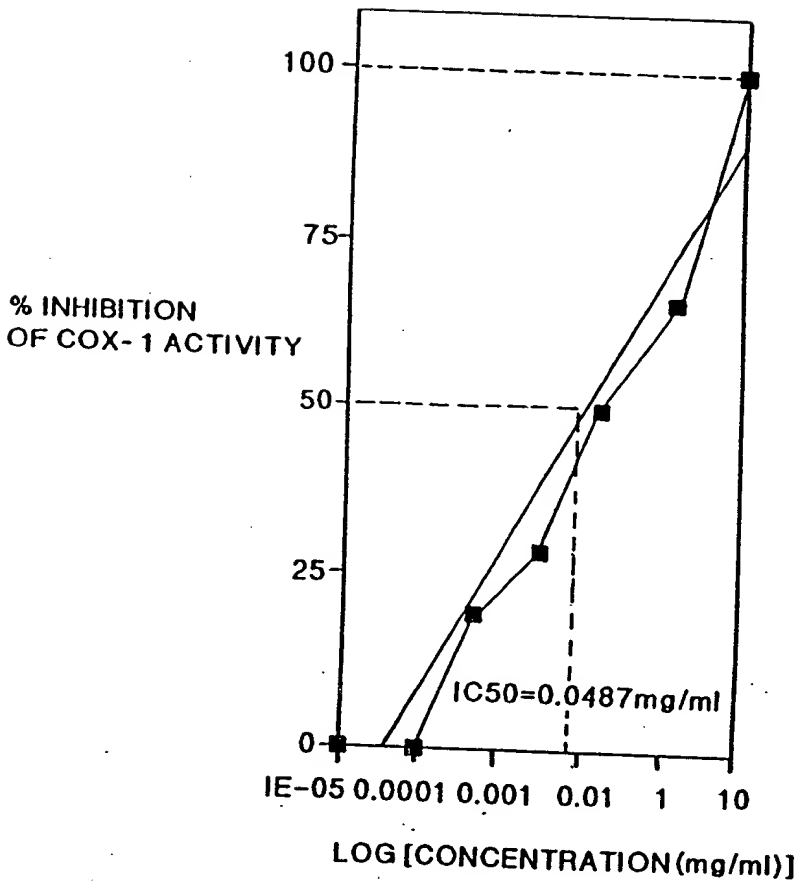

100
75
50
25
0
% INHIBITION
OF COX-1 ACTIVITY
IC50=0.0487mg/ml
IE-05 0.0001 0.001 0.01 1 10
LOG [CONCENTRATION (mg/ml)]

FIG.18U

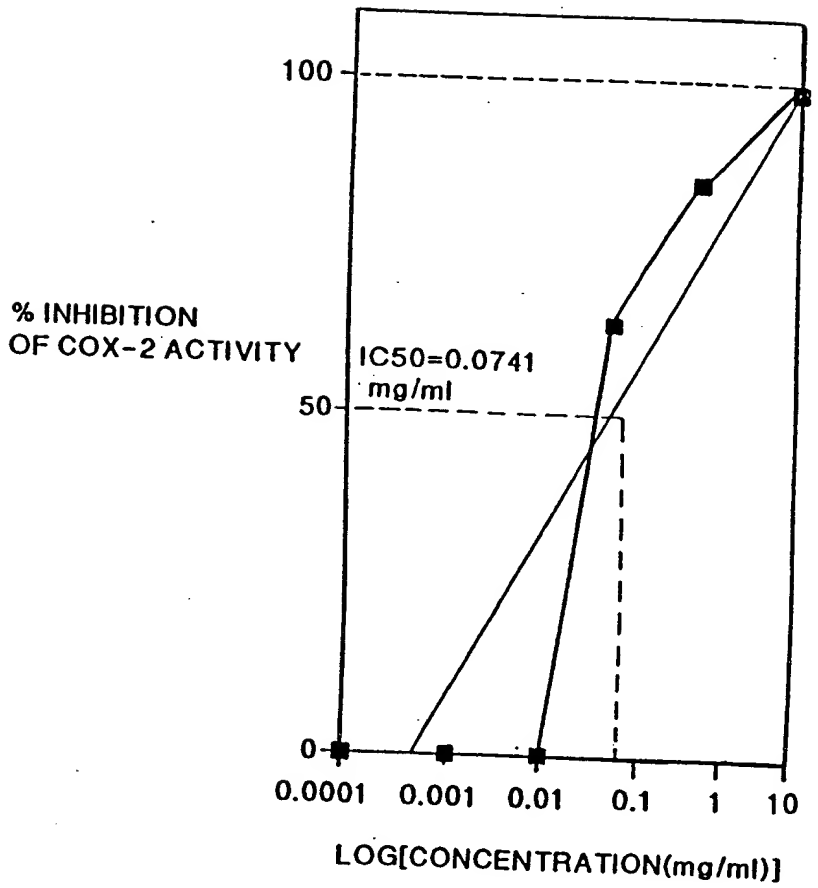

100
50
0
% INHIBITION
OF COX-2 ACTIVITY
IC50=0.0741
mg/ml
0.0001
0.001
0.01
0.1
1
10
LOG[CONCENTRATION(mg/ml)]

FIG.18V

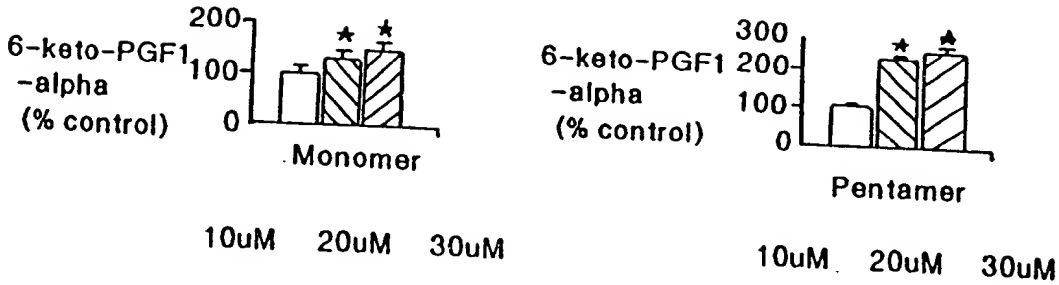


FIG.19A

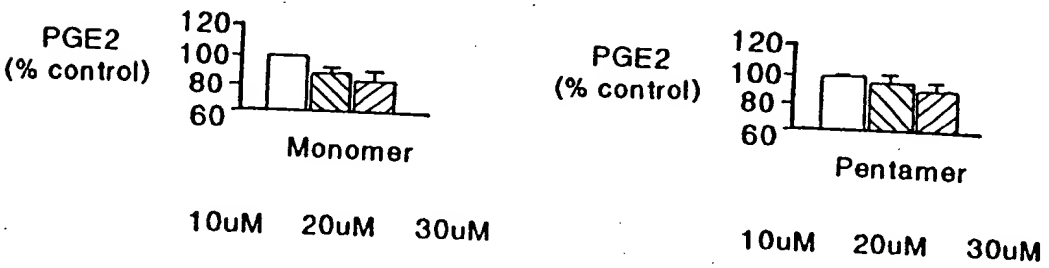


FIG.19B

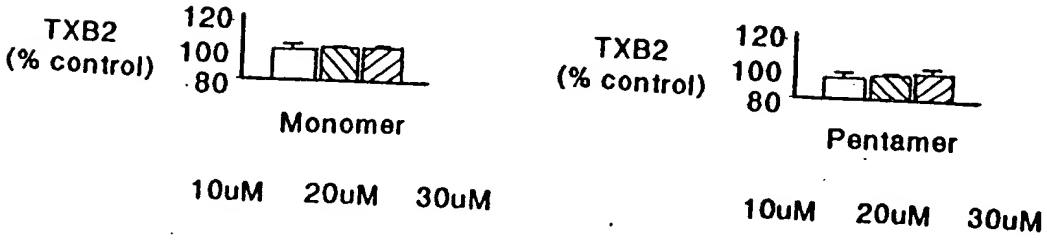


FIG.19C

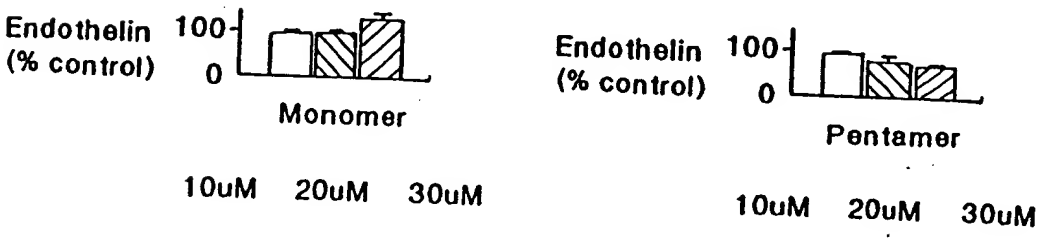


FIG.19D

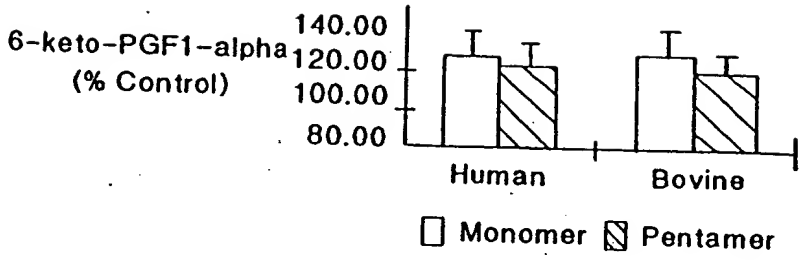


FIG.20A

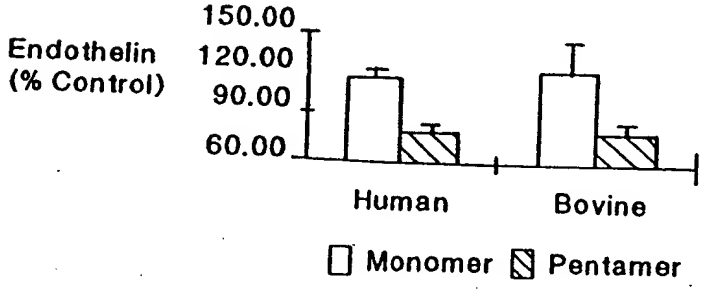
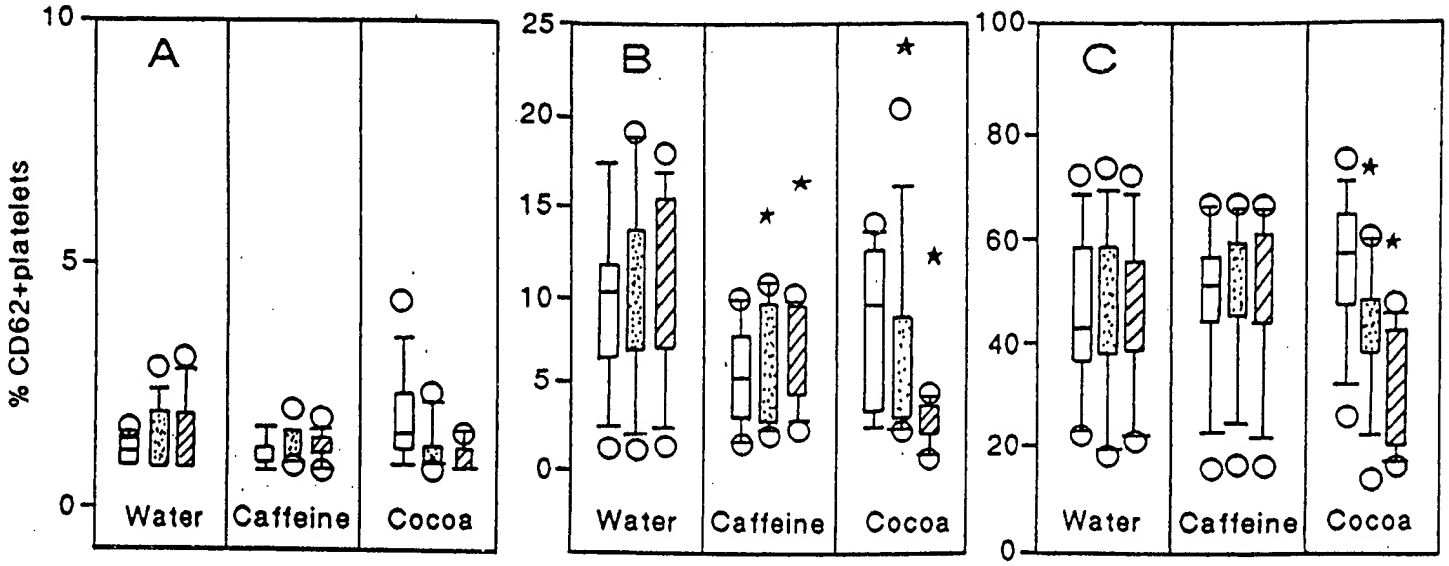


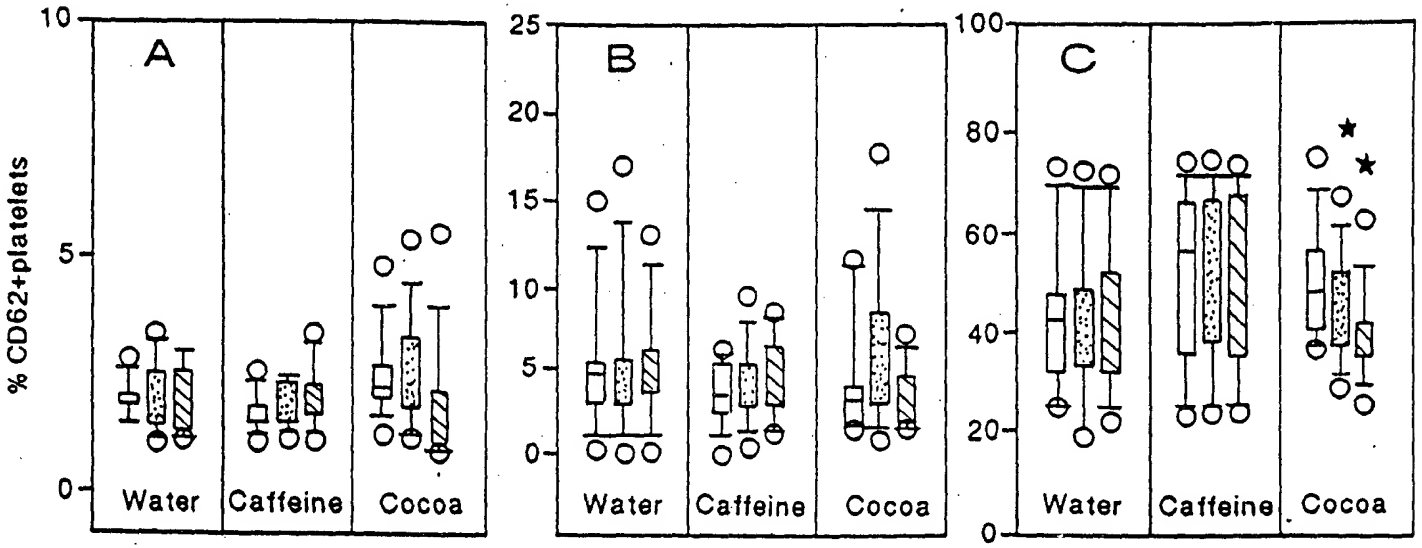
FIG.20B

FIG.21



Effect of cocoa beverage consumption on platelet surface expression of activated GP11b-111a with and without simulation with weak agonisys. Platelet activation marker expression is presented as Tukey box plots at times zero (white boxes), 2 hours (light grey boxes), and 6 hours (dark grey boxes) post consumption of water, a caffeine-containing control beverage (caffeine) or a cocoa beverage (cocoa). (A) percentage of platelets expressing activated gp11b-111a (PAC1= platelets) without stimulation (B) after stimulation with epinephrine (20uM) or (C) with ADP (20uM). Activated GP11b-111a is expressed on the surface of activated platelets. Each box showsthe 25-75th percentile, the horizontal bar in the box shows the median. . The lines outside the box show the 10th and 90th percentile. Asterisks indicate P 0.05 betweenzero time and 6 hour hour time points of each respective data set repeated measure ANOVA on ranks, Student-Newman-Keuls multiple comparison method, n=10 in each

FIG.22



Effect of cocoa beverage consumption on platelet surfac surface expression of activated P-selection with a without stimulation with weak agonists. platelet activation marker expression presented as Tukey box plots at tin zero (white boxes), 2 hours (light grey boxes) and 6 hours (dark grey boxes) post-consumption of water, a caffeine-containing control beverage (caffeine) or a cocoa beverage (cocoa).(A) Percentage of platelets expres P-selection(CD62P+platelets) without stimulation, (P) after stimulation with epinephrine (20uM) or (C) with ADP(20uM). P-selection is expressed on the surface of activated Asterisks indicate P<0.05 between zero time a hours and between zero time and six